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Research Article

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Media optimization and growth parameters for the production of L-asparaginase enzyme from bacterial isolate

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ABSTRACT

The current study was aimed at L-asparaginase production and optimization using one of the promising bacterial isolates from soil. The isolate was grown on different media compositions, varied parameters like growth temperature, media pH etc and checked for L-asparaginase enzyme production. The enzyme was analyzed and the maximum enzyme activity was observed to be 2.54IU/ml, which was obtained from media composition having 2% glucose as carbon content, incubation temperature of 35^{0}C at a media pH of 7.4 and an incubation time of 24hrs at 150 rpm on a rotary shaker incubator.

Keywords: Optimization, Process parameters, Acute Lymphoblastic Leukemia, Media composition, L-asparaginase.

INTRODUCTION

L-asparaginase is therapeutic enzyme acting on L-asparagine and a promising product for the treatment of Acute Lymphocytic Leukemia. It is associated with the property of depleting the circulatory pool of L-asparagine by asparaginase catalytic action. L-asparaginase received increased attention in recent years for its anticarcinogenic potential. Cancer cells differentiate themselves from normal cells in diminished expression of L-asparagine [**1, 2**].The need for anticancer drugs is increasing and the demand to produce the enzyme L-Asparaginase in large economic quantities has become essential to meet the clinical requirements [**3**].

L-asparaginase does not occur naturally in humans. It is found in bacteria, plants and animals including guinea pigs; hence microbial source will be ideal for the potential enzyme production. Wide ranges of microbial population such as filamentous fungi, yeast and bacteria have proved to be beneficial source of this enzyme [**4-6**]. The present study was undertaken to optimize the media and process parameters to produce L-asparaginase enzyme. One of the bacterial isolates of RVCE soil samples, which produced L-asparaginase through screening method [**7]** was chosen for the current study. Many authors have published their scientific work such as production of L-asparaginase from various fermentation methods [**8**].L-Asparaginase production from microbial source through fermentation has been a unique method owing to its cost effectiveness and eco-friendly nature [**9**]. Enzymatic properties such as optimum temperature, pH and the effect of temperature on the stability of L-asparaginase II from *E. coli* MTCC 739 was determined and the purified protein showed an optimum activity at 37 °C and pH 6 [**10**].

The current research work is aimed at optimization studies of the bacterial isolate and its ability to produce the enzyme L-asparaginase.

EXPERIMENTAL SECTION

In the current study, selection of suitable production media, choosing the right inoculum parameters, pH and incubation temperature were studies. All the studies and the experiments were carried out in triplicates, the shaker speed was kept constant at 150 rpm and the basic media composition was $Na_2HPO_4.2H_20$, 6g; KH_2PO_4, 3g; NaCl 0.5g; Glucose, 1g; L-asparagine, 5g; 1M $MgSO_4.7H_20$, 2 ml; 0.1M $CaCl_2$ $2H_20$, 1 ml, distilled water up to 1000ml. The inoculum was kept for 16 -18 hrs in all the experimental trials.

Biological Strain

The strain is isolated from the soil samples of RVCE. The screened bacterial isolate [**Fig 1**] that produces L-asparaginase was employed for the present study. The isolate was sub cultured on nutrient agar media slants and incubated at 35-37^oC for 24 hr. These slants were sub cultured at monthly intervals and stored at 4^oC in the refrigerator for further research use.

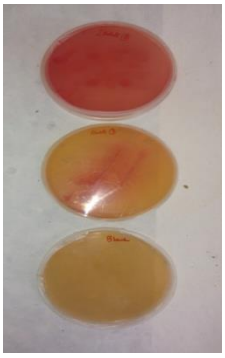

Fig 1: Plates showing production of L-asparaginase by microorganisms

Inoculum development

The bacterial growth culture of 24-hr slant was suspended in 5 ml sterile water and transferred to 45 ml of sterile inoculum medium. The composition of inoculum medium is $Na_2HPO_4.2H_20$, 6g; KH_2PO_4, 3g; NaCl 0.5g; Glucose, 1g; 1M $MgSO_4.7H_20$, 2 ml; 0.1M $CaCl_2$ $2H_20$, 1 ml; and distilled water up to 1000ml, pH 7.2. The media was incubated at 35- 37^0C for 16-18hrs. The inoculum was ready for transfer to production media.

Production process

Four different media compositions were used for the current study. Normally, glucose is chosen as a carbon source; however, it was replaced with 2% alternate carbon compounds like sucrose, maltose and lactose. Variable volumes of inoculums 5%, 7.5%, 10%, 12.5% and 15% were added to the production media and checked for the maximum L-asparaginase activity. The composition of production medium in (g/L): $Na_2HPO_4.2H_20$, 6; KH_2PO_4, 3; NaCl 0.5; Glucose, 1; L-asparagine, 5; 1M $MgSO_4.7H_20$, 2 ml; 0.1M $CaCl_2$ $2H_20$, 1 ml. The conical flasks were incubated [**Fig 2**] at 25-45^oC on a rotary shaker for 24 hrs. After the fermentation time, the broth was collected and centrifuged at 8000rpm for 10 mins. The supernatant was assayed for the enzyme activity. The pH of the production media was varied from 7.0 to 8.0. And different incubation temperatures of 25, 30, 35, 40 and 45^0C were tested for the present study.

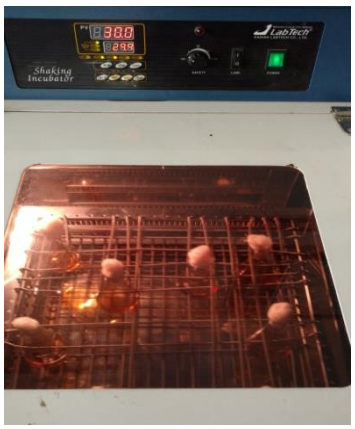

Fig 2: Optimization studies, media incubated at 30^0C

Assay for enzyme activity

For the determination of the L-asparaginase enzyme activity, Mashburn and Wriston method was followed [**11**]. In this assay, the rate of hydrolysis of L-asparagine was determined by measuring the released ammonia using Nessler's Reagent. To 100µlof supernatant obtained from the fermented broth, 200 µl of 0.05M Tris-HCl buffer (pH 8.6) and 1.7 ml of 0.01 L-asparagine were added and incubated for 10 min at 37^0C. The reaction was stopped by adding 500µl of 1.5M Trichloro acetic acid. The mixture was centrifuged at 1000rpm. 0.5ml of the supernatant was diluted with 7ml of distilled water and to this 1ml of Nessler's Reagent was added. The color reaction was allowed to develop for 10min and the OD was measured at 450 nm with spectrophotometer against blank. The ammonia liberated was read from ammonium sulphate standard curve. One unit (IU) of L-asparaginase was defined as the amount of enzyme which liberates 1µmol of ammonia per minute under the assay condition of pH 8.6 at 37^0C.

RESULTS AND DISCUSSION

The bacterial strain chosen from the soil isolate is represented in [**Fig3]**. The isolate with pink colonies were selected for the study.

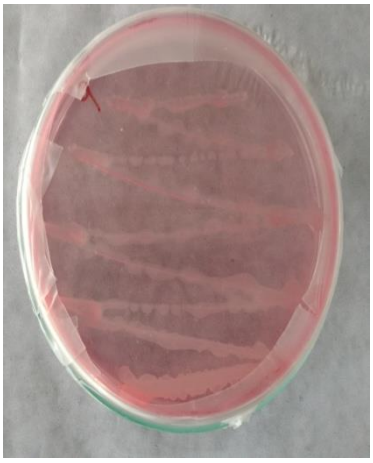

Fig 3: Bacterial isolate with pink colored colony

Media studies

The enzyme activity was checked with different media compositions, varying carbon sources. The carbon content was fixed at 2% in the media composition as shown in **[Fig 4].** The maximum activity of 2.20 IU/ml was observed with glucose at 2% concentration, incubation temperature at 35^0Cand inoculum volume at 5%.

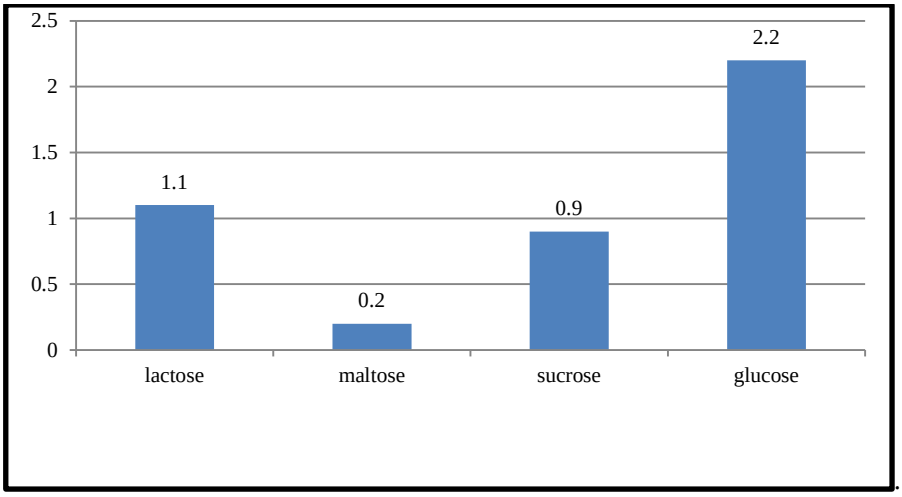

Fig 4: Media vs Activity (IU/ml)

In the subsequent studies, the glucose content was varied as 1%, 1.5%, 2.0%, 2.5%, 3.0%, and 3.5%in the media composition as shown in **Fig 5**.The maximum activity of 2.12 IU/ml was observed at 2 % glucose concentration in the media composition.

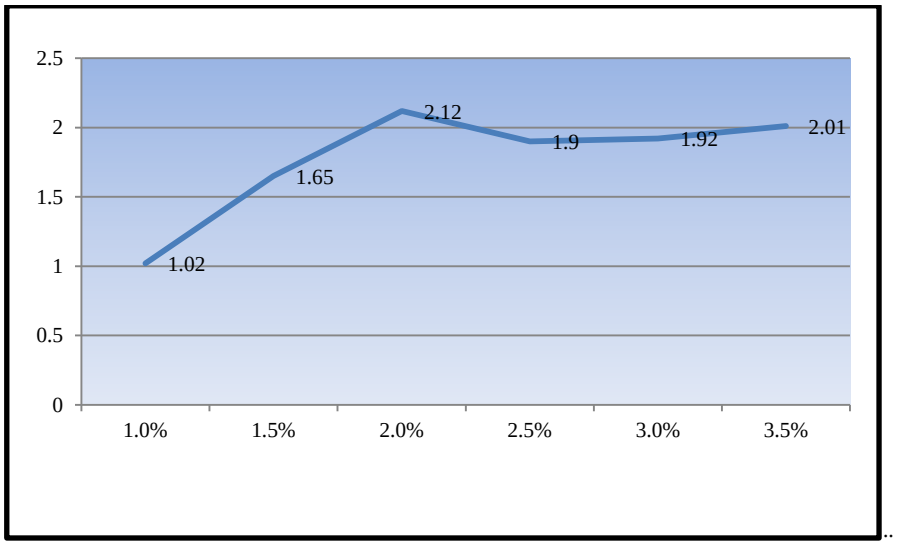

Fig 5: Activity (IU/ml) vs Glucose content (%) in media

Inoculum studies

Varied inoculum volumes of 5%, 7.5%, 10%, 12.5% and 15 %, as represented in **Fig 6**were used for the study and the maximum activity of 2.20 IU/ml was observed at10% inoculum volume to the production media.

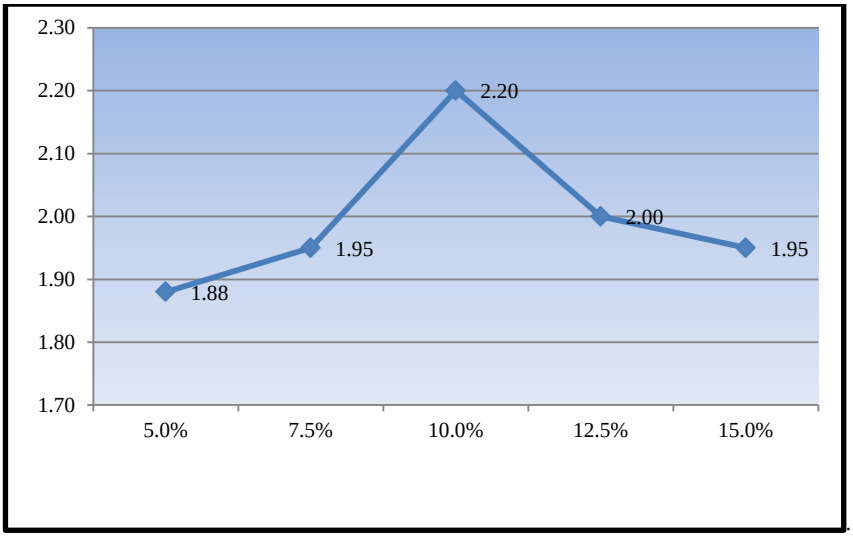

Fig 6: Inoculums % vs Activity (IU/ml)

Incubation Temperature

The experiment was conducted with different incubation temperatures as indicated in **Fig 7.** The maximum activity of 2.55 IU/ml was observed at 35^0C.

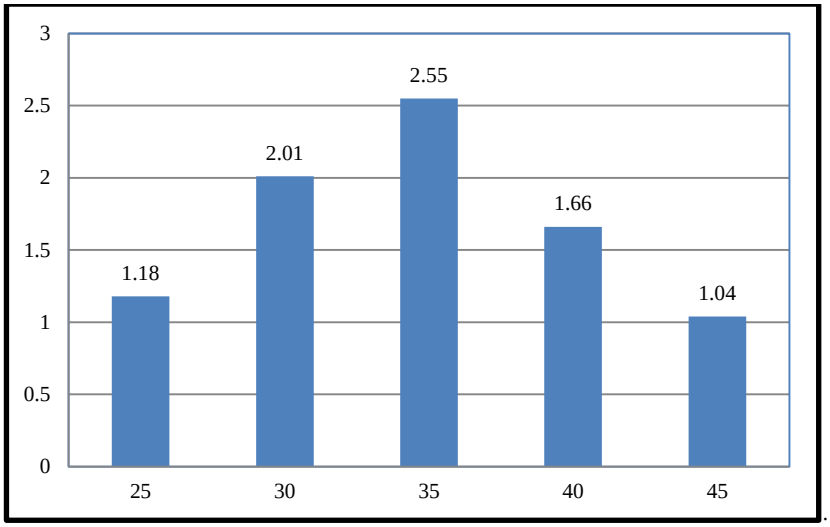

Fig 7: Incubation Temp^{0}C vs Activity (IU/ml)

Production media pH studies

The shake flask experiments were conducted at varied pH of 7.0, 7.2, 7.4, 7.6, 7.8 and 8.0. The maximum activity of 2.54 IU/ml was observed at pH of 7.4 as represented in **Fig 8.**

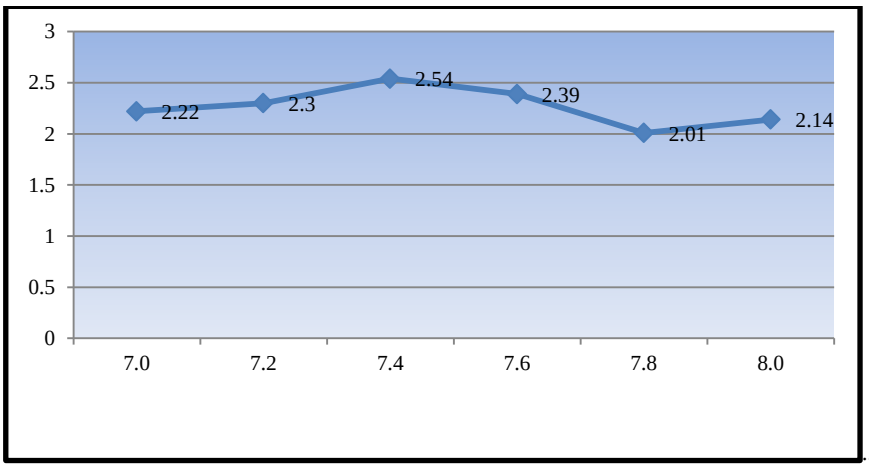

Fig 8: pH vs Activity(IU/ml)

CONCLUSION

Based on the experiments conducted on the bacterial isolate from RVCE, the best media composition, the ideal pH, the appropriate inoculum volume and the optimum incubation temperature to obtain maximum L-asparaginase enzyme production was inferred. The production media composition (in g/L: $Na_2HPO_4.2H_20$, 6; KH_2PO_4, 3; NaCl, 0.5; Glucose, 2; L-asparagine, 5; 1M $MgSO_4.7H_20$, 2 ml; 0.1M $CaCl_2$ $2H_20$, 1 ml, distilled water up to 1000ml); media pH of 7.4; inoculum volume of 10% and incubation temperature of 35^{0}C gave the maximum yield of L-asparaginase enzyme at 2.54 IU/ml .Further higher scale experiments will be conducted to optimize the process parameters.

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