ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Study the Effect of Some Growth Factors on the Production of L-Asparaginase Enzyme by Fungal Isolate *Aspergillus flavus*

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Abstract

Nine filamentous fungi from infection fruits and vegetables .Seven isolated were of high productivity for L-Asparaginase enzyme compared with other isolates, this isolates were *Aspergillus flavus, A.niger, Pencillium sp., Fusarium sp., Aspergillus flavus* was the best isolate for Asparaginase production, depending on diameteron pink colour on the medium plates. Studied the effect of nitrogen and carbon sources, and PH the resulted revealed that the maximum Asparginase production was the following condition: PH 6.0, Glocose as a carbon source, Ammonium sulphate was (121.28) U/100ml.

Introduction

"L-Asparaginase is one of the most important biomedical and biotechnology group of enzyme as it constitutes nearly (40%) of the total worldwide enzyme sales" [1].It is present in many organisms including animals, plant, microorganisms and in the serum of certain rodents but not in humans" [2]."L-Asparaginase from microbial sources has gained much attention because of its high productivity it is extracellular and there for secreted in to the fermentation medium. Among microbes this enzyme is produce by Bacteria, Fungi and Actinomycetes L-Asparaginase from eukaryotic microorganisms is gaining much importance as it is known to have less adverse effects" [3].

"Microbial L-asparaginase are preferred because microorganisms produce a bund ant amounts of the desired product in a short period of time and can be easily manipulated through genetic engineering to generate more stable enzymes with altered properties than other sources" [4]."Filamentous fungi are one of the most important Asparaginase sources for industrial application because fungal enzymes are usually excreted extracellular, facilitating extraction from the fermentation media with low cost and high productivity and are more resistant to harsh climatic condition" [5]. Optimization of nutritional and physical requirements of microorganism is important to develop and control the the economic feasibility of any bio-process.

"The optimum levels of process parameters for maximum enzyme production are unique for each microorganism. In this concern no defined medium has been established for the optimum production of L-asprginse from different microbial sources therefore, the aim of present study was to screen new potent fungal isolatates and microorganisms processing extracellular L-Asparginase production capacity in addition, optimization of cultural and environmental condition required for enzyme production will be carried out for the highest L-Asparaginase" [6].

Materials and Methods

The fungi used throughout this study, *Fusarium sp*., *Aspergillus niger*, *A. fluvus* ,*Pencillium sp*. And *Alternaria alternate*.and denification by phenol and microscopic characters, all strains were grown on incubation (Potato dextrose agar) slants at (30) C^0 for 6 days and used as inoculums. This fungal isolation by many infection fruits and vegetables .Were collected from Mosul markets/ Iraq.

Production of (L-Asaraginase) by Fungi

"The fungal culture was maintained on (Potato Dextrose Agar) slant at 4c and sub cultured on (PDA) plates in cubated at (30)c for 6 days and used as inoculums ,the culture medium used for the study was modified (czapek-dox) medium containing g/L: Glucose

(2.0), L-Asparagine (10), KH_2PO_4(1.52), KCL(0.52), $MgSO_4.7H_2O$(0.52), $CuNO_3.3H_2O$ (trace), $ZnSO_4.7H_2O$ (trace), $FeSO_4.7H_2O$ (trace), PH=6.0 [6], the flasks were Autoclaved (15) min at a pressure of (16/in ch^2). Cultivation was achieved by adding (2ml) of the fungal suspension previously prepared as a standard inoculums in (100) ml of the fermentation medium placed in (250) ml, and then incubated at (30) C^0 in a reciprocal shaker (200) rpm for 3 days.

The extracellular enzyme was prepared by centrifugation at (500) rpm for (20) min growing culture of *Pencillium sp*. *Alternaria alternate*, Fusarium *sp*. And *Aspergillus niger* and *Aspergillus fluvus* (two flask for each isolate). Then the flask were without inoculation was used as control" [7].

Effect of PH on Enzyme Production

To exam the effect of PH on maximum enzyme production the medium was adjusted to various PH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5). Prior to autoclaving and the organism was inoculated the flasks were incubated for (6) days and estimated the activity of enzyme.

Effect of Carbone Sources on Enzyme Production

"In present study different carbone sources were added to modified (Zapek-Dox's) liquid media at equivalent weight. Various sources of carbon such as soluble (Fructose, Lactose, Dextrose, Maltose, Glucose).Were supplemented with L-Asparagine (0.3) % as nitrogen sources in growth media. The inoculums was added in the medium and incubated at (31) C^0 for 6 days" [7].

Effect of Different Nitrogen Sources on Enzyme Production

"As affect the nitrogen sources considering the secondary energy source after carbon sources and they play a vital role in the growth of organisms and enzyme production .In microorganisms, Amino acid, nucleic acid, Protein .In the present experiment the supplementation of additional nitrogen sources such as (Urea, Pepton, Ammonium nitrate, Yeast extract and Ammonium sulphate) "[8].

Results and Discussion

Fungal Isolate

"Different techniques were used for isolation and characterization the fungi and done with help morphology. The total numbers of isolated fungi were (11) strain showed in the Table (1).All isolates were identified using morphological and microscopic characterization" [9].

Basal Medium used for the Qualitative Production of Enzyme

The fungi isolated from infection samples (fruit and vegetables) a simple screening for L-Aparaginase production by agar plate assay as reported by [10]. There are total [6]. Fungal species and isolates gave positive test with variable degrees depending upon the intensity of the produced pink color (Fig.1).

The formation of pink color can be interpretedby the breakdown of amide bond in L-Asaragine by L-Asparaginase with accumulation of Ammonia in the medium Aspergillus niger, *A. fluvas*, *Pencillium sp*., *Fusarium sp*., exhibited pink color. While *Alternaria alternate* gave negative test. My results were same with study of [11].

(A) (B)

Fig.1: Showing a pink colour around the organisms (B) *Aspergillus fluvus* (A) *Penicillium sp*. were grown on modified (Czapek-Dox agar) plate

Table 1: Different fungi found on samples and Asparaginase Production from fungal isolates

No.	Place of isolation	Fungi	Result
1	Apple	*Aspergillus niger*	+
2	Strawberry	*Aspergillus .fluvus*	+++
3	Orange	*Fusarium sp.*	+
4	Potato	*Penicillium sp.*	++
5	Cucumber	*Alternaria alternate*	–
6	Tomato	*Alternaria alternate*	–
7	Apple	*Penicillium sp.*	+
8	Tomato	*Aspergillus niger*	+
9	Potato	*Fusarium sp.*	+

L-Asparginase Produce by Various Fungal

The results from Table (2).Showed the best produces was asparagenase that getting from *Aspergillus flavus*. Asparaginase activity was (110.36) U/100ml other fungal isolated seen to be as a weak Asparaginase activity was (52.70) U/100 ml from *Fusarum sp*. So in this study we used A. *fluvas* to Asparaginase produce. Show Fig. (2).

Table 2: Asparaginase produce by various fungal isolates

Fungal isolated	*A*sparaginase activity U/100ml
Aspergillus fluvas	**110.36**
A.niger	**84.53**
Penicillium sp.	**100.68**
Fusarium sp.	**52.70**

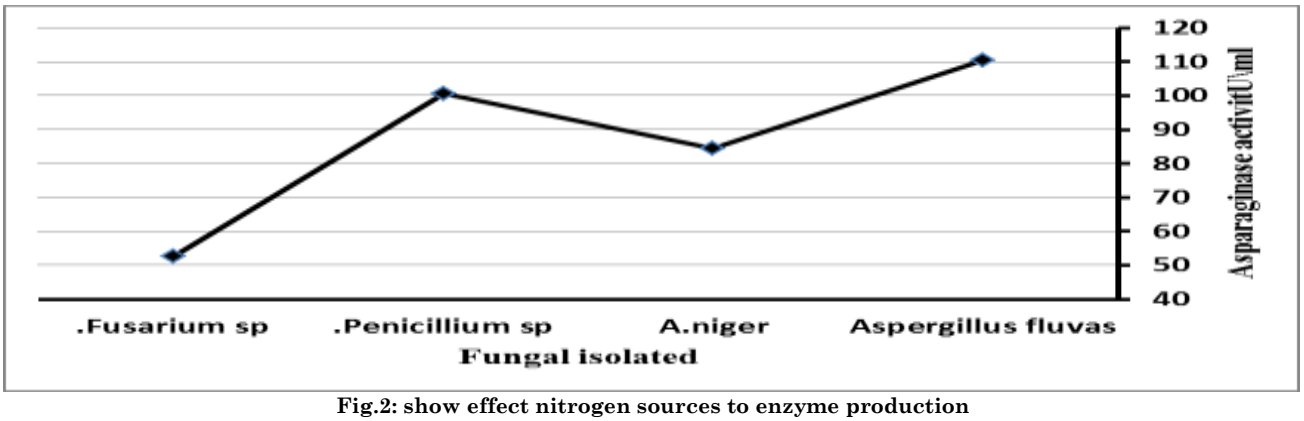

Fig.2: show effect nitrogen sources to enzyme production

Effect of Carbon Source to L-Asparganinase Enzyme Production

From Table (3) above .The L-Asparaginase activity was best yield in media has supplemented with glucose (112) U/100ml compared with Maltose having medium gave (72) U/100ml as inhibited enzyme production, Fig (3). Similar results have been reported in literature. [12]. Hence the glucose was used for further optimization studies [13].This results are similar with study [14].

Table 3: Effect of different carbon sources on L-Asparaginase

Carbon sources	Enzyme activity U/100ml
Maltose	**72.82**
Lactose	**80.60**
Glucose	**112.67**
Fructose	**86.66**
Dextrose	**93.54**

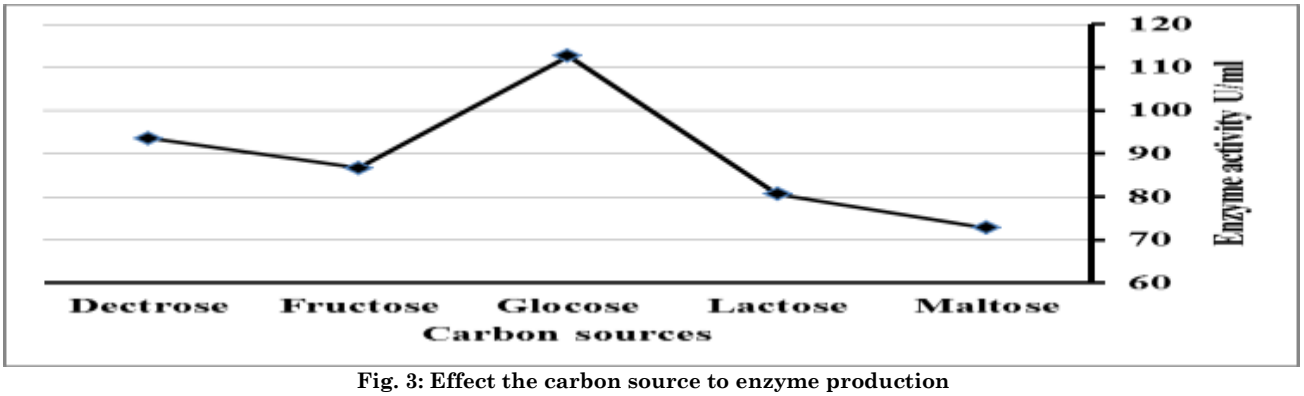

Fig. 3: Effect the carbon source to enzyme production

Effect of PH to L-Asparaginase Production

"The enzyme activity can be either enhanced or inhibited depending on the change in the PH, and hence can in flsence the growth of microorganism" [15].Different organisms have different PH, optima and any modification higher L-Asparaginase activity (107.4) U/100ml was obtained at PH (6.0), Table and Fig. (4), while least enzyme production (59.70) U/100ml was observed at PH (4.0) Fig (4). "The maximum enzyme activity of *Pencillium sp.*, was at PH (6.0) the culture significantly in flnence mamy enzymatic process and transport of the compounds across the cell membrane" [16]. Studied similar results for the production of L-asparginase by *Aspergillus niger* and *A.terreus* respectively [17]. And also similar results were reported by [18].For L-Asparginase production by *Aspergillus flavus* and (*Penicillium brevicompactum*).

Table4: Effect of PH on L-Asparaginase production

PH	enzym activity U/100ml
4.0	59.70
4.5	63.87
5.0	71.88
5.5	89.09
6.0	107.7
6.5	91.76
7.0	83.91

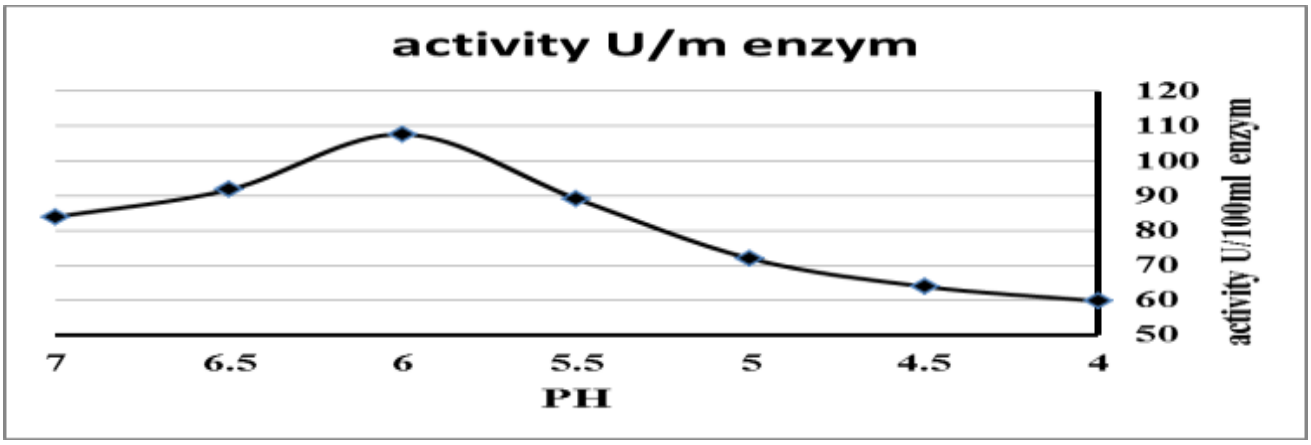

Fig.4: Effect the different PH of the enzyme production

Effect of Different Nitrogen Sources of the Enzyme Production

The results in table, Fig. (5), shows to the high produce from L-asparaginase was (118.85) U/100ml in the culture inclued (Ammonium sulphate) compared with other nitrogen sources which had an inhibitory effect on the enzyme. (Ammonium sulphate) test that supported the growth and production of enzyme"[19, 23].While [24] was found the best nitrogen source (Ammonium chloride) for L-Asparaginase production by *Aspergillus terreus*.

Table5: Effect the nitrogen source to enzyme production

Nitrogen source	Enzyme activity U/100ml
Peptone	66.43
Ammonium sulphate	121.28
Ammonium chloride	78.00
Potassium nitrate	90.31
Sodium nitrate	71.80

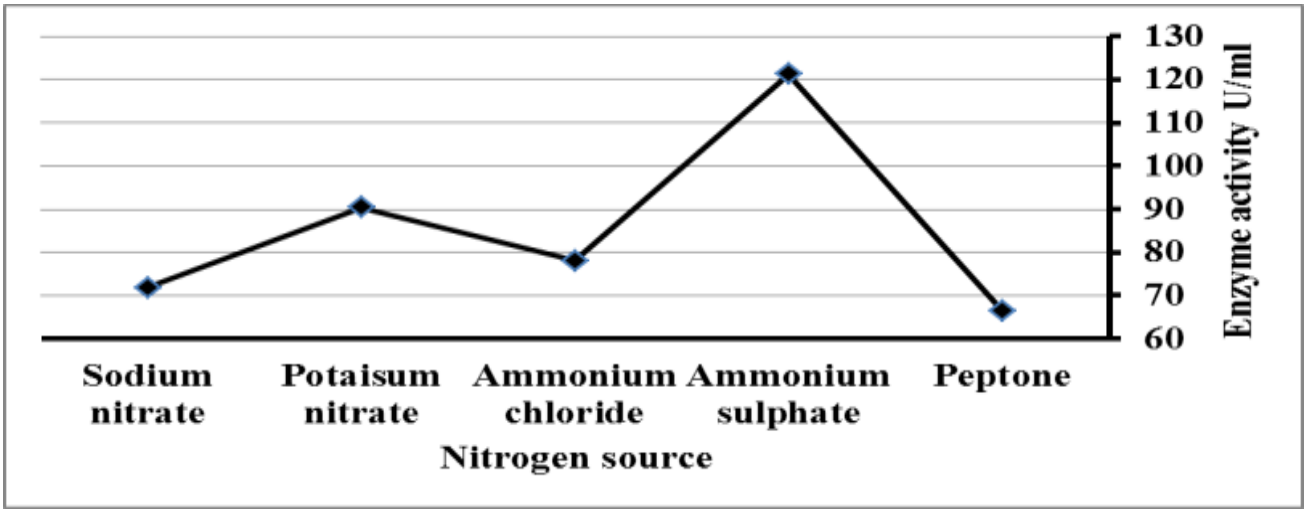

Fig.5: Effect the nitrogen sources to enzyme production

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