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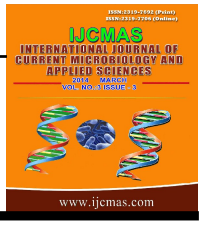

Original Research Article

Antitumour property l-glutaminase on from *Aspergillus oryzae* through submergrd fermentation

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Keywords

L-glutaminase; antitumor property; MTT assay; MCF-7 (breast cancer cell line).

A B S T R A C T

L-Glutaminase, an amidohydrolase enzyme has been a choice of interest in the treatment of lymphoblastic leukemia. Accumulating evidences suggest the beneficial effects of amino acid-depleting enzymes in lowering the risk of various cancers. *Aspergillus oryzae* strains were isolated and screened by plate assay method. *Aspergillus oryzae* S2 evolved as potential strain and it showed 217.65 IU. Then the L-glutaminase were tested for their antitumour property by employing MTT assay against the human cell line named as MCF-7 (breast cancer cell line) and their IC_{50} is 283.288 ug/ml.

Introduction

Enzyme industry is one among the major industries of the world and there exists a great market for enzymes in general. Enzymes are in great demand for use in several industries, such as food, beverage, starch and confectioneries production as well as in the textile and leather processing, pharmaceuticals and waste treatment. There is an increasing demand for microbial enzymes such as cellulose (Bajpai, 1999), ·-galactosidase (Clark et al., 2000).

Microbial enzymes are routinely used in many industrial sectors, as they are economic and environmental friendly. Microbial enzymes are preferred over plant or animal sources due to their economic production, consistency, ease of process modification and optimization. Among the major human diseases cancer is the most dangerous diseases. It is a second bigger disease of human beings. Although several kinds of treatments are available, enzyme therapy is equally effective.

L-asparaginase and L- glutaminase (L-Glutamine amidohydrolase EC 3.5.1.2.) earned attention since the discovery of their antitumor properties (Broome, 1961; Bauer et al., 1971; Abell and Uren, 1981. L-Glutaminase (L-glutamineamido hydrolase EC 3.5.1.2) catalyzes the

hydrolysis of L glutamine to L-glutamic acid and ammonia (Nandakumar et al., 2003; in recent years, L- glutaminase has attracted much attention with respect to proposed applications in both pharmaceuticals and food.

A variety of microorganisms, including bacteria, yeast, moulds and filamentous fungi have been reported to produce L-glutaminase (Kashyap*et al.,* 2002, Weingand-Ziade *et al.,* 2003 and Iyer and Singhal 2008) of which the most potent producers are fungi (Balagurunathan*et al.,* 2010). On an industrial scale, glutaminases are produced mainly by *Aspergillus*and *Trichoderma*sp (Tomita *et al.,* 1988, Masuoa*et al.,* 2004, El-Sayed 2009 and Palem*et al.,* 2010).

The objective of this study was to utilize *Aspergillusoryzae*with good ability to produce L-glutaminase and purify the enzyme, and purified enzymes were used for checking the antitumour property of L-glutaminaseagainst the human cell line named as MCF-7 (breast cancer cell line).

Materials and Methods

Chemicals

Glutamine used in the study was procured from Hi-Media Laboratories, Mumbai, India; the other ingredients used for the preparation of CzapekDox's media were also products of Hi-Media Laboratories, Bombay.

Fungal strain

The *Aspergillus oryzae* strains were isolated from different soils. Soils are taken from different regions from Vijayawada. Tentatively identified in the laboratory and further the strains were identified at Agarkar research Institute (ARI), Pune.

Fermentation medium and fermentation conditions for L-glutaminase biosynthesis

Aspergillus oryzae S2 were cultured on production medium. The production medium consists of dextrose 0.1%, yeast extract 0.3%, KCl 0.02%, NaCl, 0.01%, MgCl2 0.02% and starch 0.5% w/v.The fermentation conditions of the production medium is pH 5, temperature 35^0C and inoculum size were kept as 1.0 ml.

Assay of L-glutaminase

Assay of L-glutaminase was carried out as per Imad et al., (1973). L-glutamine were used as a substrate and the product ammonia were released during catalysis and it was measured by using Nesseler's reagent. The enzyme activity was expressed in International unit (IU).

Antitumour activity of L-glutaminase by MTT assay

The anti-proliferative effect of the purified L-glutaminase enzyme on tumor human cell line named as MCF-7 (breast cancer cell line), was assessed by the mitochondrial dependent reduction of yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to purple formazan (Mosmann, 1983), [(plate -1(a) and plate-1(b)].The MTT assay method described bellow.

1. Seed $5x10^3$ MCF7 cells in 100µl DMEM medium with 10% FBS per well in a 96 well plate and incubate overnight at 37℃/5% CO_2.

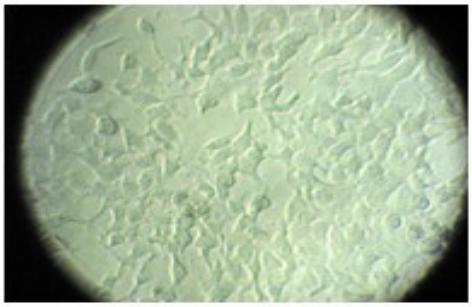
Table. 1. MTT Assay of L-glutaminase using MCF7 cell line

	optical density					
concentration	1st	2nd	3rd	mean OD	mean OD - blank	% of inhibition
blank	0.072	0.053	0.069	0.064667		
dox	0.088	0.074	0.054	0.072	0.007333	99.14563107
no treatment	0.944	0.958	0.867	0.923	0.858333	
0.5mg/ml	0.276	0.219	0.21	0.235	0.170333	80.15533981
0.25mg/ml	0.481	0.477	0.479	0.479	0.414333	51.72815534
0.1mg/ml	0.691	0.679	0.768	0.712667	0.648	24.50485437
0.05mg/ml	0.847	1.051	0.848	0.915333	0.850667	0.893203883

Plate.1 MTT Assay of L-glutaminase

Fig 1a:With out L-Glutam

(i) No Treatment (-ve control) (ii) Doxorubicin hydrochloride Treatment (+ve)



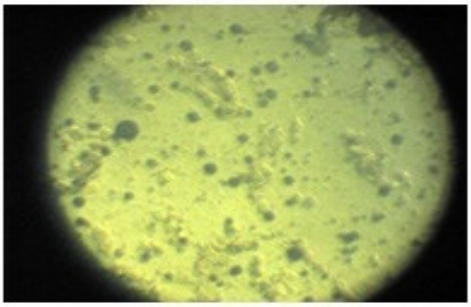
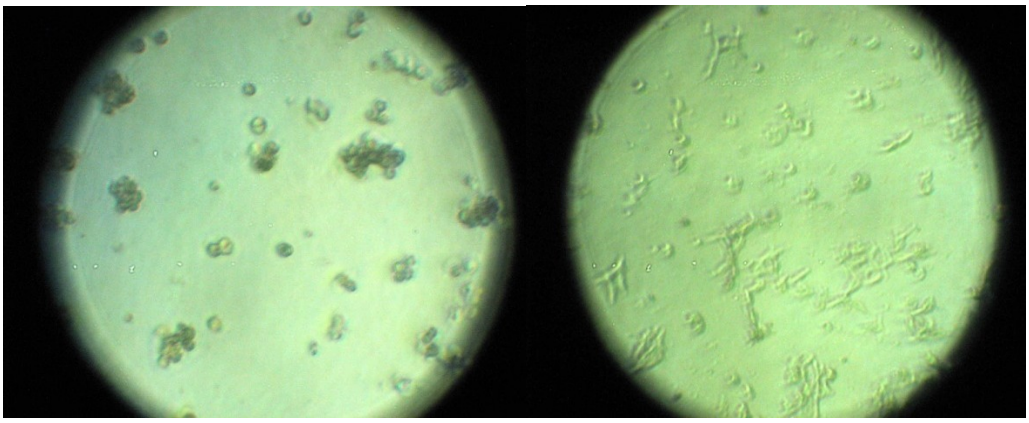


Fig :1b. L-Glutaminase treated

- a.Dose applied 0.5mg/ml b. Dose applied 0.25mg/ml



2. Prepare 1mg/ml stock of the test samples in DMEM. From this stock serially dilute the sample in DMEM-10% FBS.
3. Add 100 µl of these diluted samples to cells in triplicate wells containing 100 µl of medium.
4. 100 µl of DMEM was used as negative control and 5µg/ml Doxorubicin was added as internal positive control for the assay. Wells without any cells are used as blank.
5. Mix and incubate at 37℃/5% CO_2 for 72 hours.
6. Add 20µl of 5mg/ml MTT in PBS to each well and incubate at 37℃/5% CO_2 for 4 hours.
7. Aspirate the medium and add 200 µl of Dimethyl sulfoxide (DMSO) to each well.
8. Measure the optical density of each well using Microplate reader at 570 nm.

Inhibition of growth is a measure of cytotoxicity and the percentage inhibition is

Calculated as follows:

% Inhibition (% cytotoxicity) = 100- [(Mean OD for test sample/mean OD for the Control) x100]

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration lethal to 50% of the cells (IC_{50}).

Results and Discussion

Fungal isolates were identified as *Aspergillusoryzae*in Agrakar Research Institute, Pune. All twenty five strains of *Aspergillusoryzae*produced pink zones on glutamine plate medium; those were selected from the soil sample. Of the twenty five isolates *Aspergillusoryzae*S2 was considered to be the best and high L-glutaminase producing strain. The maximum enzyme activity was showed at 217.65 IU.

Using MTT assay, the in vitro bioassay cytotoxic effect of *Aspergillus oryzae* S2L-glutaminase on the growth of human tumor cell line-MCF-7 [Breast cancer cell line] showed that the purified enzyme extracts have anti-proliferative activity [Table-1, plate-1(a) and plate-1(b)].

Inhibition of growth is a measure of cytotoxicity and the percentage inhibition is calculated as follows:

% Inhibition (%cytotoxicity) = 100 - [(Mean OD for test sample/mean OD for the Control) ×100]

The plot of %cytotoxicity versus sample concentration was used to calculate the concentration lethal to 50% of the cells (IC_{50}). MTT assay showed that the given sample of L-glutaminase is toxic to MCF7 cells and IC_{50} for Sample L-glutaminase is 283.288 ug/ml. In this connection, the cytotoxicity of L-glutaminase from *Aspergillus flavus* KUGF009 towards MCF-7 cell lines by the MTT assay (IC50 250 µg/ml) was reported by Nathiya *et al*. (2011) and Elshafei (2014) et al reported purified L-glutaminase IC_{50}63.3 µg/ml and partially purified L-glutaminase is IC_{50}109.9 µg/ml. Our results are good closely agrees with Nathiya et al. (2011)

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