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# A Promising Technique for Rapid Screening and Confirmation of L-Glutaminase - A Tumour Inhibitor from Novel *Pencillium expansum*

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**ABSTRACT**
All fourteen isolates of *Pencillium expansum* were screened for the production of L-glutaminase. The screening of L-glutaminase producing isolates carried out by using modified Czapek Dox's agar plate. Out of fourteen isolates the strain *P. expansum* KGSSD 08 were showed high and potential L-glutaminase producer. It showed maximum 1.11cm zone of diameter. Then the rapid confirmation of L-glutaminase producing *Pencillium expansum* KGSSD 08 were carried out by thin layer chromatography and the Rf Values were determined. The Rf value is 0.268.This Rf was close to that of standard glutamic acid.

**Keywords:** L-glutaminase, plate assay, *Pencillium expansum*, thin layer chromatography.

## INTRODUCTION

L-glutaminase (L-glutamine amidohydrolase E.C 3.5.1.2) is the enzyme that catalyzes the deamidation of L-glutamine to L-glutamic acid and ammonia. [1] L-glutaminase has received significant attention since it was reported extensively as antileukemic agent. [2-4] Unlike normal cells, leukemic cell do not demonstrate the L-glutamine synthetase, thus it dependent on the exogenous supply of L-glutamine for their growth and survival. [5]

Tumour cells have an absolute requirement for glutamine as a growth substrate. Glutamine is required as a precursor for both DNA synthesis and protein synthesis, and may also be used as a respiratory substrate. In experiments where glutamine metabolism in tumour cells has been specifically compared with that in non-transformed cells of the same origin, glutamine metabolism in the tumour cells has been found to be considerably faster. This is true for human hepatocytes and hepatoma cells. [6]

L-Glutaminase has received significant attention recently, owing to its potential applications in medicine as an anticancer agent and in food industries. [7-8] Microbial glutaminase have found applications in several fields. It has been tried as therapeutic agents in the treatment of cancer [1, 10] and HIV. [9] It is also used as an analytical agent in determination of glutamine and glutamate. [11] However one of the major use of microbial glutaminase in the food industry as a flavor enhancing agent. [12]

L- glutaminase is generally regarded as a key enzyme that controls the delicious taste of fermented foods such as soy sauce. [13]

To our knowledge, the reports on production of L-glutaminase from *P. expansum* are scanty. In the present study an attempt was made to produce L-glutaminase using a locally isolated strain of *P. expansum* from soil. The work also focuses on the development of promising technique for rapid detection and confirmation of the isolated strains by plate assay and thin layer chromatography.

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## MATERIALS AND METHODS

**Chemicals:** L-glutamine used in the study was procured from Hi-Media Laboratories, Bombay, India and other ingredients used for the preparation of Czapek Dox's media were also products of Hi-Media Laboratories, Bombay. Silica Gel G for thin layer chromatography and Phenol for the solvent system was procured from SRL Laboratories, India.

**Organisms:** The *Pencillium expansum* strains were isolated from different soils. Various soil samples were collected from different region of Bangalore university campus.

**Medium:** The organisms were grown and kept on slants of solid modified Czapek Dox's medium containing (g/L distilled water) glucose, 2; L-glutamine 10; KH2PO4, 1.52; KCl, 0.52; MgSO4.7H2O, 0.52; CuNO3.3H2O, trace; ZnSO4.7H20, trace FeSO4, trace; agar, 20.0

Modified Czapek Dox's medium was supplemented with different concentrations of the dye. A 2.5% stock of the dye was prepared in ethanol and the pH was adjusted to 7.0 using 1M NaOH. The stock solution of the dye ranging from 0.04 ml to 0.3 ml was added to 100 ml of modified Czapek Dox's medium, giving final dye concentration of 0.2% with a final pH of 7.0. The media were autoclaved and plates were prepared, control plates of modified Czapek Dox's medium were (i) without dye and (ii) without glutamine. [14]

The plates were then inoculated with 96 h cultures of *Pencillium expansum* for rapid screening of glutaminase. The diameter of the colonies and the diameter of total clear hydrolytic halos including the colonies were determined. The strains that yielded higher halos were selected as potential microorganisms for L-glutaminase production using glutamine as substrate. Percent of cleared zone was calculated according to the formula given below.

Percent of cleared zone =

$$\frac{\text{Diameter of cleared zone - Diameter of colony}}{\text{Diameter of colony}} \times 100$$

## RAPID CONFIRMATION OF L-GLUTAMINASE BY TLC METHOD

Primarily screened strains were subjected to thin layer chromatography (TLC) for the confirmation of L-glutaminase production. Here the separation and identification of amino acids were carried out by thin layer chromatography technique as modified method of Arima *et al*. [15] by using silica gel G and saturated phenol with water used as a solvent system The enzyme activity, or an amount of aspartic acid produced was roughly estimated by redness of the spot developed by spraying ninhydrin reagent.

## RESULTS AND DISCUSSION

In the present study fourteen isolates of *Pencillium expansum* (Plate-1) were isolated from different soil samples and named serially from KGSS1 to KGSS14. This screening of filamentous fungi is based on the semi qualitative method described by Gulati *et al*. [14] The results from plate assay are presented in plate-2. The grouping of strains of *Pencillium expansum* has been done on the basis of zone of diameter they exhibited for convenience.

It was proposed that the strain exhibiting zone of diameter above 2.0 to 3.0 cm were referred to as good or high L-glutaminase producers, those strains with zone of diameter 1.0 to 2.0 cm and those having below 1.0 cm zone of diameter may be referred to as moderate and poor L-glutaminase producers respectively. As per the grouping of the strain, *Pencillium expansum* KGSS08 exhibited higher zone of diameter (3.3 cm) and was considered as a potential strain of L-glutaminase producer among the strains isolated from soil. These results were close agreement with the reports of Gulati *et al* [14] and Siddalingeshwara *et al*. [16] An attempt was also made to score the percentage of cleared zone. Here the *Pencillium expansum* KGSS08 were exhibited 57.142% of cleared zone.

The results on the confirmation of L-glutaminase production from the isolates using TLC are presented in Plate-3 and Table 1. To ensure whether the zone diameter exhibited by *Pencillium expansum* strains on rapid plate assay method was due to L-glutaminase activity, the glutamic acid formed due to catalysis of L-glutamine was subjected to TLC for further confirmation.

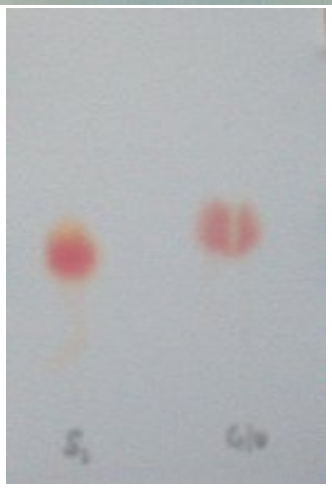
**Table 1: Confirmation of L-glutaminase production from *Pencillium expansum* by TLC method**

| S. No. | Sample | Rf Values |
|---|---|---|
| 1. | Standard glutamic acid | 0.27 |
| 2. | *Pencillium expansum* KGSS08 | 0.268 |

**Plate 1: *Pencillium expansum***

**Plate 2: Rapid plate assay for screening of L-glutaminase producers**

**Plate 3: Confirmation of L-glutaminase production from *Pencillium expansum* by TLC method**



**$S_1$- L-glutamine Catalyzed by L-glutaminase for the formation of glutamic acid and $S_2$- Standard glutamic acid**

The TLC technique was used for the separation and identification of glutamic acid produced by *Pencillium expansum* and was roughly estimated by redness of the spot developed by spraying ninhydrin reagent. The glutamic acid is a compound produced, after the hydrolysis of glutamine by glutaminase enzyme synthesized by the *P. expansum* KGSS08 strains. In this study, the compound produced by the isolates exhibited similar Rf values (0.268) as that of standard glutamic acid (0.27).These results were similar to that observed with filamentous fungi Siddalingeshwar *et al*. [16] To our knowledge this is the first attempt to confirm L-glutaminase production by *Pencillium expansum* KGSS08

strains by thin layer chromatography and percentage of cleared zone.

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