International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.2, pp 830-833, April-June 2010

Rapid Screening and Confirmation of L-Glutaminase producing Novel *Aspergillus wentii*

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ABSTRACT: *Aspergillus wentii* 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 twenty one isolates the strain *Aspergillus wintii* KGSD4 were showed high and potential L-glutaminase producer. It showed maximum 1.3cm zone of diameter. Then the rapid confirmation of L-glutaminase producing *Aspergillus wintii* KGSD4 were carried out by thin layer chromatography and the Rf Values were determined. The Rf value is 0.265.This Rf were close to that of standard glutamic acid.
Key words: L-glutaminase, plate assay, *Aspergillus wentii*, thin layer chromatography.

INTRODUCTION

L-Glutaminase has received significant attention recently owing to its potential applications in medicine as an anticancer agent and in food industries [1, 2]. Microbial glutaminases have found applications in several fields. They had been tried as therapeutic agents in the treatment of cancer [3,4] and HIV[5] as an analytical agent in determination of glutamine an glutamate[6]. However one of the major uses of microbial glutaminase is in the food industry where it is used as a flavor enhancing agent[7]. L-Glutaminase is generally regarded as a key enzyme that controls the delicious taste of fermented foods such as soy sauce[8].

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

MATERIALS AND METHODS

CHEMICALS:

L-glutamine used in the study was procured from Hi-Media Laboratories, Bombay, India; the 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 *Aspergillus wentii* strains were isolated from different soils. Soils are taken from different region from 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 1 mol L-1 NaOH. The stock solution of the dye ranging from 0.04 ml to 0.3ml 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 prepared, control plates were modified Czapek Dox's medium (i) without dye and (ii) without glutamine. The plates were then inoculated with 96 hr cultures of *Aspergillus wentii* for rapid screening of glutaminase. The zone and colony diameters were measured after 48 hr for *Aspergillus wintii*.

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., 1972[9] 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 ninhydrin reagent.

RESULTS AND DISCUSSIONS

In the present study tweenty one isolates of *A. wentii* (Plate-1) were isolated from different soil samples and named serially from KGSD1 to KGSD21. This screening of filamentous fungi is based on the semi qualitative method[10]. The results from plate assay are presented in plate-2. Therefore for convenience the grouping of strains of *A. wentii* has been done on the basis of zone of diameter they exhibited. It was proposed that the strain exhibiting zone of diameter above 1.0 to 1.5 cm were referred to as good or high L-glutaminase producers, those strains with zone of diameter 0.6 to 0.9 cm and those having below 0.6 cm zone of diameter may be referred to as moderate and poor L-glutaminase producers respectively. As per the grouping the strain *A. wentii* KGSD4 exhibited higher zone of diameter (1.3 cm) and were considered as a potential strain of L-glutaminase production among the strains isolated from soil. These results were similar to that observed with *A. terreus* [10,11].

The results on the confirmation of L-glutaminase production from the isolates using TLC are presented in Plate-2 and Table-2. To ensure whether the zone of diameter exhibited by *A. wentii* strains on rapid plate assay method was due to L-glutaminase activity, the enzyme extract was subjected to TLC for rapid confirmation. The TLC technique was used for the separation and identification of glutamic acid produced by *A.wentii* strains were 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 *A.wentii* KGSD4 strains. In this study, the compound produced by the isolates exhibited similar Rf values (0.265) as that of standard glutamic acid (0.27).These results were similar to that observed with filamentous fungi by Thany & Thana 2006, Lingappa & Siddalingeshwar 2004. To our knowledge this is the first attempt to confirm L-glutaminase production by *A.wentii* KGSD4 strains by thin layer chromatography.

TABLE- 1: CONFIRMATION OF L-GLUTAMINASE PRODUCTION FROM *Aspergillus wentii* BY TLC METHOD

Sl. No.	Sample	Rf Values
1	Standard glutamic acid	0.27
2	*Aspergillus wentii* SD4	0.265

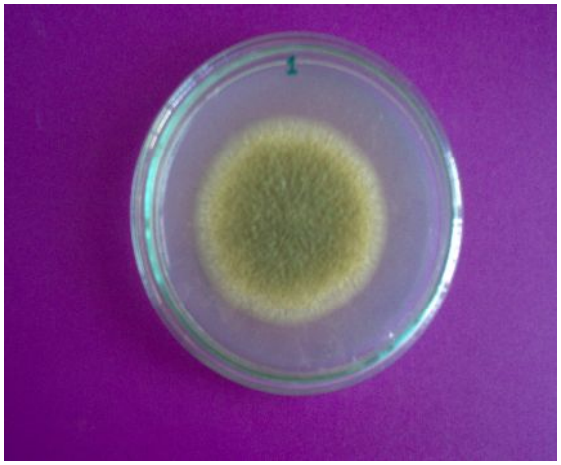


Plate-1 *Aspergillus wentii*

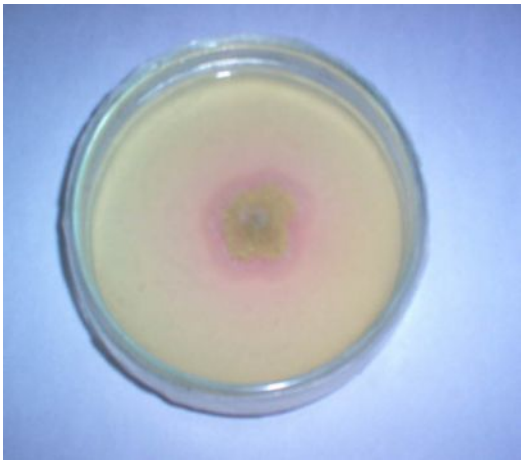
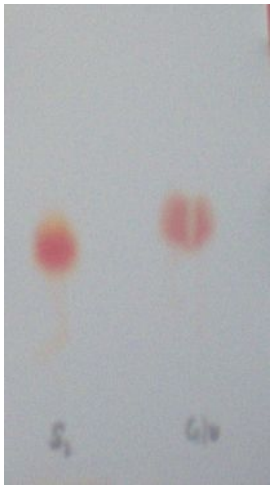


Plate-2 Rapid plate assay for screening of l-glutaminase producers



S_1 **S_2**

PLATE 3: CONFIRMATION OF L-GLUTAMINASE PRODUCTION FROM *Aspergillus wentii* BY TLC METHOD

S_1- Catalyzed production of glutamine by L-glutaminase
S_2- Standard glutamic acid

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