Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 5, Issue 4, 2013

Research Article

COMPARATIVE STUDY OF AMINO ACIDS PRODUCTION BY *ASPERGILLUS WENTII* IN DIFFERENT CULTURE MEDIA

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Received: 13 July 2013, Revised and Accepted: 22 July 2013

ABSTRACT

Aspergillus wentii is filamentous fungi that multiplied in optimum condition in Czapex Dox and Glucose ammonium media then extracellularly produced amino acids in submerged culture condition. These amino acids were identified by TLC and compared to Rf values of standards. In this study we identified amino acids in varying incubation periods.

Keywords: *Aspergillus wentii*, Thin Layer Chromatography, Incubation period and Amino acids.

INTRODUCTION

A*spergillus wentii* is filamentous fungi and during growth phase it produced extra cellular amino acids in their culture filtrate in different incubation time intervals. They are nitrogenous compound and serve as energy source. Amino acids are the building blocks of proteins. They bound together in chains and formed the stuff and also formed peptides or polypeptides. Twenty different kinds of amino acids are required for growth of fungi. Therefore they synthesized by *Aspergillus wentii* and secrets in their culture filtrate. Commonly recognized amino acids including are Glutamine, Glycine, Phenylalanine, Tryptophan, Isoleucine, Leucine, Lysine, Methionine, Threonine and Valine. Three amino acids i.e. Phenylalanine, Tryptophan, and Valine are essential amino acids for humans and cannot be synthesized by the body. Hence, they must be ingested through food. *Aspergillus* culture were grown in two different media and incubated for different incubation time then extracts were used for identification of amino acids by TLC using ninhydrin reagent. Rf values of samples were compared with Rf values of standards.

REVIEW OF LITERATURE

Thirteen amino acids were isolated from culture and culture filtrate of *Aspergillus* (Woolley and Peterson, 1936; 1937a & *b*). Amino acid was determined by ninhydrine reaction (Sobel, Hirschman and Besman, 1945).The concentration of amino acids to be depending on age of organism and environment condition (Stokes and Gunness, 1946; Lugg, 1949; Sueoka, 1961; Holden, 1962; Rao & Ventkataraman, 1952; Meyers & Knight, 1961; Chattaway, Toothill & Barlow, 1962).

Meyers & Knight (1961) reported that conidia and mycelium of *P. roqueforti* showed the same qualitative patterns of free amino acids. The amino acid composition of fungal mycelium and spores is not well known but qualitative or quantitative analyses of free amino acid in various fungi were assessed (Holden, 1962).

Amino acids were examined in culture filtrates of fungi by thin-layer chromatography using Stahl and Kaltenbach method (Stahl, E. and Kaltenbach, V., 1965). Submerged colonies of *Aspergillus* produced amino acids in their culture filtrate (Galbraith & Smith, 1969). The separation and identification of amino acids were carried out by thin layer chromatography technique as modified method of Arima *et al.* (1972) by using silica gel G and saturated phenol with water used as a solvent system. Lysine has been recognized as one of the most deficient essential amino acids in the food supply of both human beings and meat producing animals because it is not synthesized biologically in the body (Pelczar *et al.,* 1993).

In certain actinomycetes fungi and algae**,** the carbon skeleton of L-lysine arises from acetate (Anonymous, 1993). Several methods have been reported for production of amino acids by fungi (Volkel, D. and Wagner, F., 1995). Fungi are involved in a variety of industries, e.g. food, chemical, detergent, textiles and paper industries where used for a variety of industrial production (Moreira *et al.,* 1999 and 2001; Kathiresan and Manivannan, 2006).

MATERIAL AND METHODS

Three soil samples were collected from Botanical garden of Rajiv Gandhi Govt. PG College, Mandsaur, Madhya Pradesh then all three samples were mixed together and formed one sample. 10.0g soil was used for isolation of fungi by serial dilution technique and prepared soil dilutions where 10^{-3} dilution was used for fungal isolation. Sample (0.1ml) was spread on potato dextrose agar (PDA) medium plates and incubated at 30°C for 5 days then again purified by streak plate method. After purification, fungal cultures were identified by microscopic method using laboratory manual for introductory mycology (Smith *et al.,* 1983).

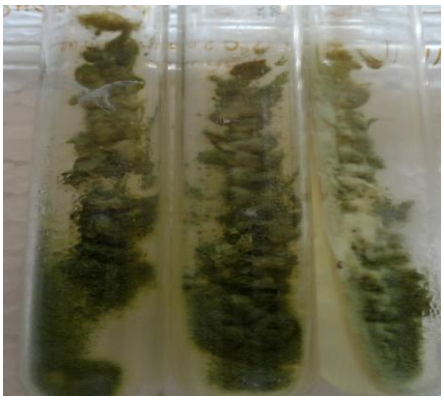
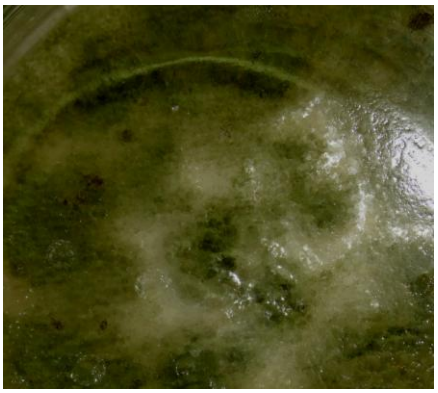


Fig. 1: a. Culture slant



b. Culture Plate

Production of amino acid in Czapek Dox and Glucose ammonium medium

Some spores of *Aspergillus wentii* were transferred on PDA slant and incubated for 5 days at 30˚C then 5.0 ml sterilized distilled water was introduced to culture slant and scrapped by wire loop. Spore suspension was transferred to 100.0ml Czapek Dox and Glucose ammonium production medium and incubated at 30˚C for 3, 5, 7 and 9 days. Culture filtrate was obtained from each day by filtration using whatman No. 1 filter paper and concentrated by evaporated at 40°C in water bath.

Fig. 2: Production medium: Left to right Czapek Dox and glucose ammonium medium.

Identification of amino acids by TLC (Thin Layer chromatography)

Prepared silica gel plates by dissolving silica gel G into D.water in 2:1 ratio and mixed vigorously by homogenizer then poured on glass plate (4 x10 cm). Plates were dried at room temperature for 24 hrs and then removed whole moisture by heating at 50°C for 48 hrs in hot air oven. Culture filtrates and amino acid standards were spotted by glass capillaries and dried then plate was inserted into TLC glass chamber having 70:30 ratio**s** of ethanol and water. TLC chamber was sealed and left for 1-2 hrs. Before passed out solvent from top of plate, it was removed from chamber and dried in hot air oven then sprayed by 0.2% ninhydrine in acetone reagent. Plate were again dried in hot air oven at 60°C for 2 hrs then calculate Rf value of spots and compared sample Rf value to standards Rf values by following formula:

$$\text{Rf value} = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by solvent}}$$

RESULT AND DISCUSSION

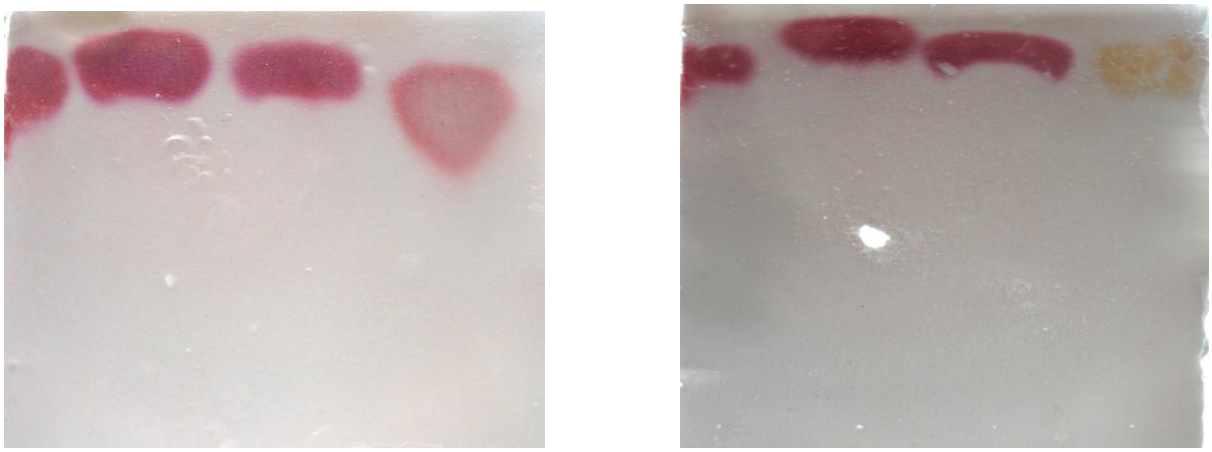
Aspergillus wentii grown in both production medium having sugar and produced amino acids in culture filtrate which was qualitatively estimated by TLC fig. 3, 4, 5 and 6 and table 1 & 2 indicated that glutamic acid, serine, cystine and valine produced in 3, 5, 7 and 9 incubation days respectively in Czapek Dox medium while glutamic acid, hydroxyl proline, leucine and glutamic acid in Glucose ammonium medium during 3, 5, 7 and 9 incubation days respectively.

Table 1: Identification of amino acids by TLC in culture filtrate of Czapek Dox medium

S. No.	Amino acid std.	Rf of std.	Day of incubation			
			3 (Rf)	5(Rf)	7(Rf)	9(Rf)
1	DL- Valine	0.983333				0.983333
2	DL-Serine	0.916666		0.916666		
3	L-Glutamic acid	0.941666	0.941666			
4	L-Cystine	0.967479			0.966666	

Table 2: Identification of amino acids by TLC in culture filtrate of Glucose ammonium medium.

S. No.	Amino acid	Standard (Rf)	Day of incubation			
			3 (Rf)	5(Rf)	7(Rf)	9(Rf)
1	L-Hydroxy Proline	0.958333		0.95967741		
2	L-Glutamic acid	0.941666	0.94354838			0.934959
3	L-Leucine	0.976190			0.9756097	



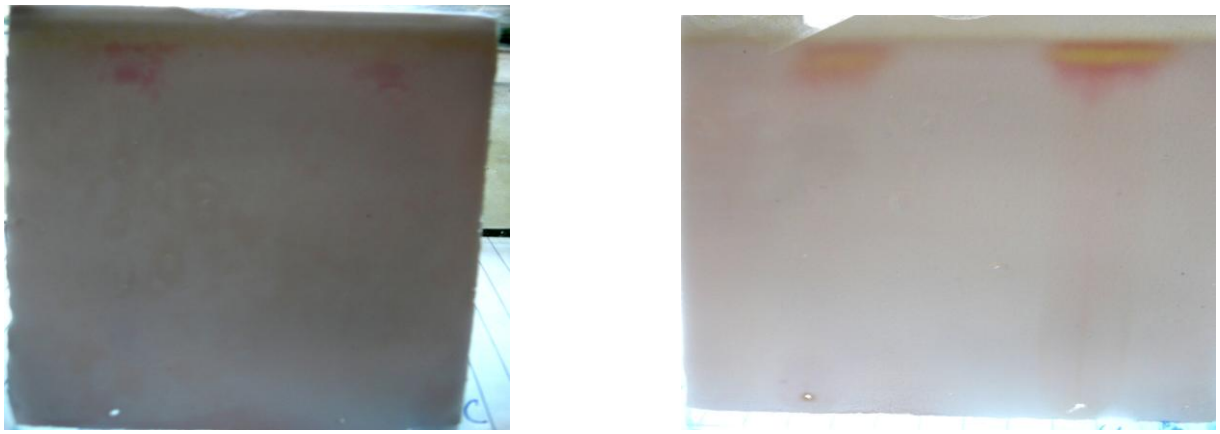
a **b**

Fig. 3: Amino acids standards: a- left to right showing alanine, valine. Threonine and glycine & b- left to right showing serine, nor-leucine, glutamic and hydroxyl proline.

a **b**

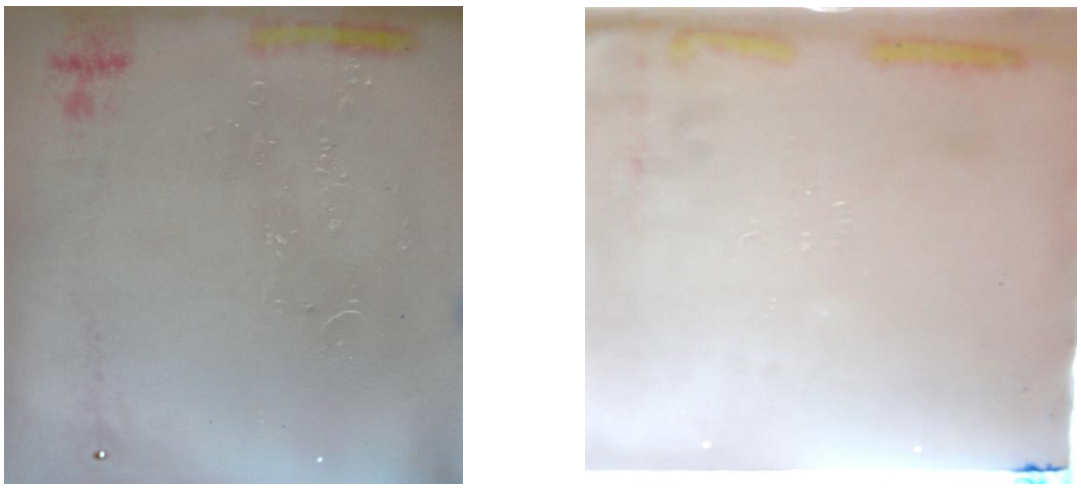
Fig. 4: Amino acids standards: a- left to right showing isoleucine and leucine & b- left to right showing cystine and amino butyric acid.

Fungi multiplied in submerged growth condition and produced spores (Galbraith and Smith, 1969) which geminated and generate hyphae then nutrient used by them and produced amino acids in expontial phase. Nakayama (1972) also suggested that amino acids were produced by microbes. For amino acid production they required sugar which was available in both production medium as sucrose in Czapek Dox and glucose in Glucose ammonium medium.



a **b**

Fig. 5: Identification of amino acid in culture filtrates of *A. wentii* in Czapex Dox medium: a- left to right 3 days & b- 5 days.



a **b**

Fig. 6: Identification of amino acid in culture filtrates of *A. wentii* in Glucose ammonium medium: a- left to right 3 days & b- 5 days.

CONCLUSION

It can be suggested that *Aspergillus* spp. and other microorganisms produced extracellular amino acids by fermentation technology which will be used as nutrient supplements specially amino acid for fastidious organisms as well as human beings.

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