

Study on Physiological and Nutritional Factors of Fungi isolated from External Ear Canal

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Abstract

The present study aimed to examine the nutritional requirements, temperature, and pH of fungi. The influence of culture medium, various ranges of temperature, and pH was analyzed on the mycelial growth of fungi. The test fungi isolated from Otomycotic patients. Three test fungi *Aspergillus niger*, *Aspergillus flavus*, and *Candida* were cultured on six different types of culture media including Sabouraud's dextrose broth (SDB), Potato dextrose broth (PDB), Czapek's Dox broth (CDB), Malt Extract broth (MEB), Tryptone soy broth (TSB) and Littman bile broth base (LBB). The fungal growth and sporulation were analyzed at various temperatures (20°C, 25°C, 30°C, 35°C, 40°C, and 45°C) and pH values (4, 5, 6, 7, 8, and 9). In the present study, Sabouraud's dextrose broth is the most suitable broth medium for all three test fungi, *Aspergillus niger* (380 mg dry mycelial weight), *Aspergillus flavus* (308 mg dry mycelial weight), and *Candida* (164 mg dry mycelial weight). The optimum temperature range is 25-35°C and the pH range is 5-7. The fungal isolates grow best at 30°C, *Aspergillus niger* (329 mg dry mycelium weight), *Aspergillus flavus* (316 mg dry mycelium weight), and *Candida* (148 mg dry mycelium weight). The maximum fungal growth observed at pH 7, *Aspergillus niger* (325 mg dry mycelium weight), *Aspergillus flavus* (311 mg dry mycelium weight), and *Candida* (128 mg dry mycelium weight). The present study highlights the suitable nutritional media, temperature, and pH is important for enhancement in the growth and sporulation of Otomycotic fungi. This study may help to understand the growth characteristics of Otomycotic fungi.

Otomycosis is a fungal infection of the external ear canal. It is a common medical problem in India and other parts of the world^{10,14}. Several environmental factors are responsible for increasing fungal infections in ears like warm, humid, and dusty climates¹.

Soil is the natural source for microbial growth and fungal spores are richly found with dust particles in the air and it acts as a predisposing factor for infection of Otomycosis disease. Hot and humid conditions are very favorable for fungal growth. The etiological agents of

Otomycosis are *Aspergillus*, *Candida*, *Mucor*, and *Penicillium*¹¹. The physiology of fungi involves growth, nutrition, metabolism, reproduction, and death². Fungi need optimum conditions for their development. The main factors that affect fungal growth are nutritional medium, pH, temperature, water activity, light, and air¹⁶. Fungal growth rate changes on different types of nutrient media⁷. Solid and Liquid media are used for the isolation of fungi. The growth media contains carbon, nitrogen sources, vitamins, minerals, amino acids for the development of mycelial growth and sporulation. Temperature and pH are the significant factors for the growth of fungi. Fungal growth and sporulation are directly influenced by various ranges of temperatures and the pH of culture media directly or indirectly affects the fungal growth. Most of the fungal growth occurs at the optimum temperature range is 20°-30°C and pH range 5-7⁵. The major purpose of this study is to evaluate the ideal nutrient media, temperature, and pH for maximum yield of fungal pathogens.

Collection of Samples :

In this study, clinical samples were collected under the aseptic condition from the outer auditory canal at the ENT hospitals Ujjain (M.P.) India. All collected samples were inoculated on Sabouraud's dextrose agar (SDA) with chloramphenicol and incubated at 28°C ± 1 for one week. After incubation, cultures were examined for fungal growth. Fungi were identified by standard monographs⁸.

Impact of Culture Media on Mycelial Growth :

The following six different types of broth culture media are used like Sabouraud's dextrose broth (SDB), Potato dextrose broth (PDB), Czapek's Dox broth (CDB), Malt Extract broth (MEB), Tryptone soy broth (TSB), and Littman bile broth base (LBB) for the fungal growth. Spore suspension of all 7-day old test fungi was inoculated on SDB, PDB, CDB, MEB, TSB, and LBB flask with cycloheximide and incubated at 28°C ± 1°C for 7 days. A set of the flask was run in duplicate. The flasks were examined for growth on alternate days. After 7 days of incubation mycelial mat of each flask was collected on preweighed filter paper (Whatman's no. 1) individually and dried at 80°C temperature. The dry mycelium weight was recorded⁹. The rate of sporulation of test fungi was determined by using standard methods^{17,18}.

Impact of Temperature and pH on Mycelial Growth :

In this study, Sabouraud's dextrose broth (SDB) was used for temperature and pH. For temperature, the flasks were inoculated with a spore suspension of all 7-day old test fungi on Sabouraud's dextrose broth (SDB) with cycloheximide in duplicate sets. After inoculation flasks were incubated at different temperatures: 20°C, 25°C, 30°C, 35°C, 40°C, 45°C for 7 days. For pH, the pH levels of potassium phosphate buffer were adjusted to 4, 5, 6, 7, 8, 9 by adding 1N HCl or 1N NaOH. Spore suspension of all 7-day old test fungi was inoculated in adjusted pH levels of Sabouraud's dextrose broth (SDB) with cycloheximide in duplicate sets and incubated at 28°C ± 1°C for 7 days. After incubation mycelium dry weight was recorded.

In the present study, fungi require some special media for their growth and sporulation. The three test fungi growth was analyzed in six different liquid culture media types i.e. Sabouraud's dextrose broth (SDB), Potato dextrose broth (PDB), Czapek Dox broth (CDB), Malt Extract broth (MEB), Tryptone soy broth (TSB) and Littman bile broth base (LBB). The results showed that Sabouraud's dextrose broth is the best growth media for all test fungi. The highest dry mycelium weight of *Aspergillus niger* is (380 mg dry mycelium weight) followed by *Aspergillus flavus* (308 mg dry mycelium weight) showed good growth and sporulation and *Candida* (164 mg dry mycelium weight) showed fair growth and sporulation on Sabouraud's dextrose broth. The average dry mycelium weight was observed in malt extract broth and the minimum dry mycelium weight was observed in the Littman bile broth base is shown in table-1 and figure-1.

In this study, the effect of temperatures (20°C, 25°C, 30°C, 35°C, 40°C, and 45°C) was

observed on the growth of test fungi as shown in table-2 and figure-2. The maximum mycelial growth and sporulation were observed at 25-35°C. The highest dry mycelium weight and sporulation were recorded at 30°C in *Aspergillus niger* (329 mg dry mycelium weight), *Aspergillus flavus* (316 mg dry mycelium weight), and *Candida* (148 mg dry mycelium weight). The poor mycelium growth and sporulation were observed at 40-45°C.

The results of the effectiveness of different pH values (4, 5, 6, 7, 8, and 9) on the growth of test fungi are shown in table-3 and figure-3. The maximum growth and sporulation of *Aspergillus niger* (325 mg dry mycelium weight), *Aspergillus flavus* (311 mg dry mycelium weight), and *Candida* (128 mg dry mycelium weight) was recorded at pH 7. As the pH range above 7, it decreases mycelial growth and sporulation of all test fungi. The inadequate growth and sporulation of all test fungi were recorded at pH 9. The mycelial growth of fungi affected at alkaline conditions in a medium.

Table-1. Average dry Mycelium weight and Sporulation of *Aspergillus niger*, *Aspergillus flavus* and *Candida* in different Growth media

Growth Medium	<i>A. niger</i>		<i>A. flavus</i>		<i>Candida</i>	
	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation
Sabouraud's Dextrose Broth	380±0.01	+++	308±0.01	+++	164±0.02	++
Potato Dextrose Broth	186±0.01	++	89±0.01	+	62±0.01	+
Czapek Dox Broth	197±0.01	++	114±0.01	++	78±0.01	++
Malt Extract Broth	226±0.01	++	106±0.01	++	94±0.01	+
Tryptone Soya Broth	163±0.02	++	115±0.005	+	56±0.02	+
Littman Bile Broth	134±0.03	+	147±0.01	++	32±0.01	-

Dry Wt. = mg, Standard Deviation = SD;

Grading of Sporulation: No sporulation = (-), Poor = (+), Fair = (++) , Good = (+++)

Table-2. Average dry Mycelium weight and Sporulation of *Aspergillus niger*, *Aspergillus flavus* and *Candida* at different temperatures

Temperature	<i>A. niger</i>		<i>A. flavus</i>		<i>Candida</i>	
	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation
20°C	184±0.01	+	162±0.02	+	87±0.01	+
25°C	278±0.01	+++	234±0.03	+++	109±0.01	++
30°C	329±0.01	+++	316±0.01	+++	148±0.01	+++
35°C	282±0.005	+++	263±0.01	++	135±0.005	++
40°C	134±0.03	++	98±0.01	+	96±0.01	+
45°C	62±0.01	+	32±0.01	-	79±0.01	+

Table-3. Average dry Mycelium weight and Sporulation of *Aspergillus niger*, *Aspergillus flavus* and *Candida* at different pH

pH	<i>A. niger</i>		<i>A. flavus</i>		<i>Candida</i>	
	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation	Dry wt. (mg)/SD	Sporulation
4	182±0.01	+	158±0.01	+	84±0.01	+
5	278±0.01	++	264±0.01	++	92±0.01	+
6	294±0.01	++	268±0.01	++	109±0.01	++
7	325±0.01	+++	311±0.01	+++	128±0.02	+++
8	198±0.01	+	164±0.02	+	96±0.01	+
9	153±0.01	+	147±0.01	+	78±0.01	+

In the present study different types of broth media, temperature and pH influence the fungal mycelial growth and sporulation of Otomycotic fungi. The optimal nutrient medium supports the growth of microorganisms¹³. The growth medium provides all necessary nutrients for the growth and sporulation of fungi¹². The fungal growth changes in different types of nutrient media⁷. The results showed that Sabouraud's dextrose broth is an excellent broth media⁴ and the Littman bile broth base showed that the minimum growth rate on Otomycotic fungi. Every fungus requires various optimum conditions for its growth. The

optimum growth temperature for the mycelial growth of fungi is 20-30°C⁵. The low temperature affects the mycelial growth whereas the optimum temperature for mycelial growth is observed between 15-30°C³. The temperature increases above 35°C it inhibits the fungal growth². In our present study, the maximum mycelial growth and sporulation were observed at temperatures 25-35°C. Most of the fungal growth occurs in acidic conditions, but some strains grow well in neutral and alkaline conditions⁵. The optimum pH range for fungal growth is 5-6. The highest mycelial growth was observed at pH 7¹⁵. In

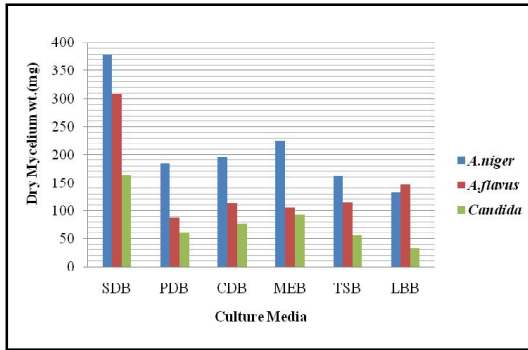


Figure 1: Influence of Culture media on the mycelium growth of *Aspergillus niger*, *Aspergillus flavus* and *Candida*

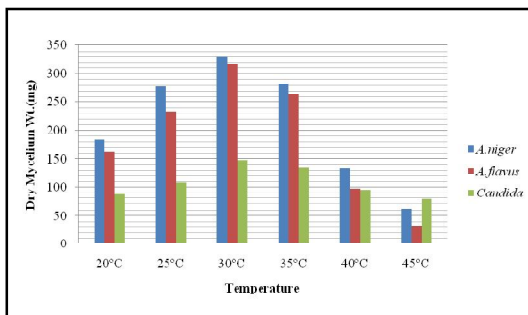


Figure 2 Effect of temperature on mycelial growth of *Aspergillus niger*, *Aspergillus flavus*, and *Candida*

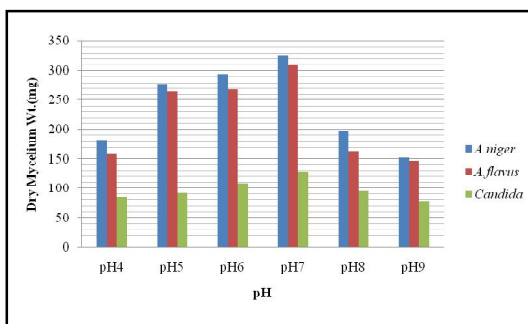


Figure: 3 Effect of pH on mycelial growth of *Aspergillus niger*, *Aspergillus flavus* and *Candida*

our present study, the maximum growth and sporulation of *Aspergillus niger*, *Aspergillus flavus*, and *Candida* was observed at pH 7.

In the present study various culture media, several ranges of temperature, and pH can influence the growth rate of fungi. The temperature and pH increase or decreases it slows the mycelial growth and sporulation. The excellent growth medium, temperature, and pH enhance the growth and sporulation of Ootomycotic fungi. The above detailed information provides a better way to understand the physiological and nutritional conditions.

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