Effect of Temperature of Incubation on the Growth, Sporulation and Secondary Metabolites Production of Aspergillus umbrosus

Manjulata Sood*
Department of Zoology, Arts & Commerce Girls College, Devendra Nagar, Raipur (C.G.)- 492004, India

Summary
Temperature is one of the most important factors influencing growth, sporulation and survival of the microorganisms. On a certain optimum temperature microorganisms will grow normally and will produce antibiotic. In this study the cultures of A. umbrosus (Bainier & Sartory) were grown on the modified Richard’s medium and then incubated at different temperature, i.e.; 15, 20, 25, 26, 30, 35, 40, 45 and 50°C for 12 days at pH 5. In respect of the incubation temperature Aspergillus umbrosus has shown a very narrow range of tolerance. Below 20°C, growth rate was very less with minimum sporulation and light yellow colouration of the medium. Optimum growth occurred at 30°C with equally good growth at 26°C. This gives a range of 26-30°C for its best growth. It tolerated temperature up to 35°C, beyond which it did not grow at all, there were no growth visualized at 40, 45 and 50°C Sporulation was also good at 26 and 30°C with dark-brown coloration of the medium, indicated the secretion of secondary metabolites. At 35°C the growth and sporulation were negatively affected and the medium colour was also greenish.

Key Words: Aspergillus umbrosus, Temperature, Incubation, Optimum, Growth & sporulation, Secondary metabolites

Introduction
Various physical factors involve under cultural conditions affect the organism for its growth and physiological performance. In all studies of fermentations much importance has been given to physical factors such as the temperature, pH, period of incubation, shaking, etc. Malama et. al. (1987) studied the kinetic of radial growth of Aspergillus colonies at various temperatures, they observed that the colonies of three Aspergillus sp. reached the highest radial growth rate at 30-40°C. At 20 and 45°C the radial growth rates were smaller than at 30-40°C. Kulshreshta and Ali (1986) have reported that A. umbrosus has possessed a good antifungal activity and could be exploited as bio-fungicide. They also observed that the fungus was a difficult organism because of very slow rate of growth and minimal sporulation. All this information about A. umbrosus evoked much interest in growth behaviour, suitable environmental and physical factors under cultural conditions.

In this study the fungus A. umbrosus has been evaluated for its optimum requirements of temperature, period and state of culture for best biomass production with good degree of sporulation and elaboration of the typical dark-brown coloration in its growth medium that is somehow associated with its antagonistic activity.

Materials and Methods
Different groups of microorganism have different optimum temperatures. Schindler et. al. (1967) studied the Aflotoxins production by Aspergillus flavus at various temperatures viz. 3, 7, 13, 18, 29, 35, 41, 46 and 52°C. They found that max. Growth occurred in at 29°C and 35°C, whereas maximum aflatoxin production occurred at 24°C, at 5 days of incubation, no aflatoxin were produced at temperature lower than 18°C and higher than 35°C and the colour of the CHCl₃ extracts appeared to be directly correlated with aflatoxin concentration. In this study the cultures of A. umbrosus were grown on the modified Richard’s medium (Broth medium) and then incubated at different temperature, i.e.; 15, 20, 25, 26, 30, 35, 40, 45 and 50°C for 12 days at pH 5, dry mycelium weight was taken as biomass, sporulation was noted and secretion of secondary metabolites creates reverse colouration in a medium was observed and further implemented for antibiotic principle.

Result
In respect of the incubation temperature Aspergillus umbrosus has shown a very narrow range to tolerance. Below 20°C, growth rate was very less with minimum sporulation and light yellow colouration of the medium. Optimum growth occurred at 30°C with equally good growth at 26°C. This gives a range of 26-30°C for its best growth. It tolerated temperature up to 35°C, beyond which it did not grow at all, there were no growth visualized at 40, 45 and 50°C Sporulation was also good at 26 and 30°C with dark-brown coloration of the medium, indicated the secretion of secondary metabolites. At 35°C the growth and sporulation were negatively affected and the medium colour was also greenish.
Discussion and Conclusions

Sorenson et al. (1967) studied the effect of temperature on the production of aflatoxin by A. flavus. They noted that the production of both aflatoxin B₁ and G₁ was found best at 28°C. At 32°C and below 28°C less amount of aflatoxin was formed. Mogenson et al. (2009) experimented the effect of temperature and water activity on the production of fumonisins by A. niger resulted that highest production of fumonisin B₂ at 25 - 30°C. Premila et al. (2006) investigated, the effect of different substrates & temperatures on the growth & aflatoxin production in A. flavus. Contaminated peanuts in Georgia were used throughout the experiment resulted that at temperature 10°C neither growth nor aflatoxin was detected, as same as 37°C. Maximum growth & aflatoxin production was attended at the temperature of 27 & 30°C in three media tested; potato dextrose agar (PDA), nutrient agar (NA) and corn meal agar (CMA). Niehaus (1989) reported that versicolorin synthesis of A. parasiticus was regulated by temperature. He suggested that a transcriptional event required for versicolorin synthesis occurred only in the presence of Zn⁺⁺ and at temperature below 37°C.

Experiments concluded with the present studies indicated that the effect of temperature in in-vitro studies on the growth, sporulation & secondary metabolites production of A. umbrosus resulted that the fungus possessed a narrow range of temperature tolerance. Optimum conditions of growth, sporulation & secondary metabolites occured at 30°C, range from 26 - 30°C, with dark-brown exudation in the medium. Below 20°C growth etc. was very low and it tolerated temperature up to 35°C beyond it the fungus did not grow at all, there were no growth visualized at 40, 45 and 50°C.

Table: - Effect of temperature on growth, sporulation & secondary metabolite production of Aspergillus umbrosus

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Temperature (in °C)</th>
<th>Growth (Biomass in mg 25ml)</th>
<th>Degree of sporulation</th>
<th>Reverse colouration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>15</td>
<td>90</td>
<td>1⁺</td>
<td>Light yellow</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>195</td>
<td>2⁺</td>
<td>Light brown</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>300</td>
<td>3⁺</td>
<td>Dark brown</td>
</tr>
<tr>
<td>4.</td>
<td>26</td>
<td>320</td>
<td>4⁺</td>
<td>Dark brown</td>
</tr>
<tr>
<td>5.</td>
<td>30</td>
<td>338</td>
<td>4⁺</td>
<td>Dark brown</td>
</tr>
<tr>
<td>6.</td>
<td>35</td>
<td>200</td>
<td>3⁺</td>
<td>Greenish</td>
</tr>
<tr>
<td>7.</td>
<td>40</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>45</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>50</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>Control</td>
<td>338</td>
<td>4⁺</td>
<td>Dark brown</td>
</tr>
</tbody>
</table>

(Modified Richard's At 30°C)

Degree of sporulation: - 1⁺ = Very poor, 2⁺ = Poor, 3⁺ = Moderate, 4⁺ = Good.
Incubation period = 12 days, pH = 5.

Fig.: Effect of temperature on growth and sporulation of Aspergillus umbrosus. Superscript on bar is degree of sporulation
References