

Effect of pH and Temperature on Three Mutants of *Aspergillus clavatus* Desm.

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The growth and development of all the microorganisms depend on a set of environmental and nutritional factors. Out of these pH and temperature are the most important. All the media and substrates upon which fungi grow in nature show the presence of hydrogen and hydroxyl ions. The pH of the medium has a remarkable effect on the rate of growth, sporulation, pigmentation and many other metabolic activities of an organism. Raper *et al.* (1953) reported that the pH of the culture medium influences the uptake of sugars from the medium. Cochrane (1958) observed that most fungi, with few exceptions, grow best on media with an initial pH of 5.0 to 6.5. Wolf and Wolf (1947) stated that the growth of many fungi stops at 0° and only a few fungi are active at 42° C, whereas the optimum temperature lies somewhere between these two.

So far there is no report regarding the effect of pH and temperature on the three mutants: albino, buff and tan of *Aspergillus clavatus* Desm. The present work was undertaken with a view to study the difference in pH and temperature requirements on the growth of these three mutants of *A. clavatus*.

Materials and Methods

The cultures of the three mutants of *A. clavatus* were obtained from Dr. V. P. Agnihotri, Mycologist, Indian Institute of Sugarcane Research, Lucknow, U. P., India. Twenty five ccm of Czapek's liquid medium was taken in 150 ccm flasks and autoclaved for 15 minutes at 15 lb pressure. These flasks were inoculated after removing the lag effect by seeding ascospore suspensions of seven days old pure cultures. In temperature experiments the flasks were incubated for 15 days at various temperatures 20° C, 25° C, 30° C, 35° C, 40° C, 45° C, 50° C and 55° C. In case of pH experiments before inoculation the flasks were steamed for three consecutive days to avoid hydrolysis. The pH range was 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. pH and was adjusted by Beckman's pH meter. These flasks were kept at 30° C for 15 days. After the incubation period, the contents of each flask were filtered through Whatman's filter paper No. 42, which were previously dehydrated and weighed to a constant weight on an electric balance.

The fungal mats were then washed with hot distilled water and the filter papers containing the mats were dried at 70° C for 72 hours. The dry weights were determined by weighing the filter papers under dry conditions to a constant weight. The filtrates were used for the determination of change in pH of the medium. The experiments were conducted in triplicates and results were statistically analysed.

Results and Discussions

The results obtained on the effect of various pH and temperatures on the growth have been given in table No. 1 and 2 respectively. The dry weight results are also graphically represented in figures No. 1 and 2. It is clear from table No. 1 that two of the mutants, albino and tan, of *A. clavatus* showed best growth at pH 6, while it was 3.5 in buff mutant. It shows that pH exerts a decided influence on the amount of growth and sporulation of fungi. Webb (1919), Johnson (1923) and Agnihotri (1964) also reported that most of the *Aspergilli* showed a better growth on the acidic side than on alkaline. The growth curves (fig. 1) of all the present mutants plotted against serial pH values showed a single maximum. Similar results have been reported by Johnson (1923), Chauhan (1953), Bhatnagar (1955) for different species of *Aspergillus*.

In view of the results obtained above, the authors support the opinion of Hawker (1957) that „No generalization can be made regarding the sporulation in relation to various pH sources“.

It is evident from fig. 2 and table 2 that the three mutants could grow between 20 to 55° C. At 30° C the growth was excellent in case of albino and buff mutants, while in tan mutant it was at 25° C. There is statistically very little difference in growth between 25 and 30° C in tan mutant and therefore 30° C can be treated as the best temperature for all the mutants. The minimum growth was recorded in albino at 55° C whereas in case of buff and tan mutant it was at 50° C. These results were in accordance with that of Thom and Raper (1945). They reported that *A. terreus* and *A. carneus* grow and sporulate luxuriantly at 30° C. Chauhan (1953) also found that temperature range of 30—35° C was best for the growth of *A. tamarii* and *A. flavus*. According to Thom and Church (1926) with the exceptions of a few thermophilic species, majority of the *Aspergilli* grow below 37° C.

The results given in table 2 showed that in general the sporulation was good, moderate, poor or nil according to the amount of growth obtained at various temperatures. Similar were the findings of Saxena and Sarbhoy (1960) in case of two species of *Cunninghamella*.

There was negligible growth recorded in control flasks in both the experiments.

Table 1
Showing the average dry weight and sporulation of three different mutants of
A. clavatus and corresponding change in pH.

| Initial pH | Albino | | Tan | | Buff | | |
|--------------------------|-------------------------|------------------|-------------------------|------------------|-------------------------|------------------|--------------------------|
| 2,5 | Dry weight in mg. | Sporu- lation | Dry weight in mg. | Sporu- lation | Dry weight in mg. | Sporu- lation | Final change of pH |
| 2,5 | 135,4 | Poor | 176,4 | Poor | 157,4 | Poor | 8,1 |
| 3,0 | 186,7 | Poor | 153,4 | Poor | 199,6 | Poor | 8,6 |
| 3,5 | 172,0 | Poor | 157,1 | Good | 217,1 | Good | 8,0 |
| 4,0 | 166,2 | Moderate | 186,6 | Good | 175,2 | Good | 8,2 |
| 4,5 | 174,8 | Poor | 182,2 | Moderate | 178,9 | Poor | 8,5 |
| 5,0 | 199,4 | Moderate | 175,1 | Moderate | 171,9 | Moderate | 8,4 |
| 5,5 | 160,3 | Moderate | 192,1 | Good | 170,6 | Good | 8,5 |
| 6,0 | 225,6 | Moderate | 195,7 | Good | 161,0 | Good | 8,2 |
| 6,5 | 159,8 | Good | 163,6 | Excellent | 184,7 | Excellent | 8,3 |
| 7,0 | 187,6 | Good | 182,7 | Good | 172,6 | Good | 8,2 |
| 7,5 | 197,6 | Excellent | 179,3 | Good | 186,6 | Excellent | 8,2 |
| 8,0 | 183,0 | Good | 179,2 | Good | 163,9 | Good | 8,3 |
| Control | Trace | | Trace | | Trace | | |
| Average dry weight | 164,0 | | 163,4 | | 164,6 | | |

Table 2
Showing the average dry weight and sporulation of three different mutants of
A. clavatus

| Tempera- ture | Albino | | Tan | | Buff | |
|------------------|-------------------|------------------|-------------------|------------------|-------------------|------------------|
| | Dry wt. in mg. | Sporu- lation | Dry wt. in mg. | Sporu- lation | Dry wt. in mg. | Sporu- lation |
| 20°C | 122,27 | ++ | 114,5 | +++ | 116,9 | ½++ |
| 25°C | 194,1 | +++ | 208,5 | ++++ | 182,7 | ++++ |
| 30°C | 218,4 | ++++ | 204,6 | ++++ | 198,4 | ++++ |
| 35°C | 171,9 | ++++ | 136,9 | +++ | 145,4 | +++ |
| 40°C | 168,5 | ++ | 147,9 | +++ | 142,3 | +++ |
| 45°C | 138,8 | ++ | 195,0 | +++ | 161,9 | +++ |
| 50°C | 119,7 | ++ | 79,2 | ++ | 82,3 | ++ |
| 55°C | 85,0 | ++ | 96,2 | ++ | 111,6 | +++ |
| Control | No growth | | No growth | | No growth | |
| Average dry | 135,4 | | 131,4 | | 126,8 | |

Summary

The best growth for two mutants of *Aspergillus clavatus* was at pH 6 whereas it was 3.5 in buff mutant. The best temperature for all the three mutants was 30° C.

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Figure 1: Showing the growth in mgs of three mutants of *A. clavatus* at different hydrogen ion concentrations.

Figure 2: Showing the growth in mgs of three mutants of *A. clavatus* at different temperatures.

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