# 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 impotant. 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 151b 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  $70^{\circ}$  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  $55^{\circ}$  C. At  $30^{\circ}$  C the growth was excellent in case of albino and buff mutants, while in tan mutant it was at  $25^{\circ}$  C. There is statistically very little difference in growth between 25 and  $30^{\circ}$  C in tan mutant and therefore  $30^{\circ}$  C can be treated as the best temperature for all the mutants. The minimum growth was recorded in albino at  $55^{\circ}$  C whereas in case of buff and tan mutant it was at  $50^{\circ}$  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  $30^{\circ}$  C. Chauhan (1953) also found that temperature range of  $30-35^{\circ}$  C was best for the growth o 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  $37^{\circ}$  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 Saksena and Sarbhoy (1960) in case of two species of Cunning-hamella.

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

| $\frac{\mathbf{Initial}}{\mathbf{pH}}$ $\frac{2,5}{2}$ | Albino                  |                  | Tan                     |                  | Buff                    |                  |                       |    |
|--|-------------------------|------------------|-------------------------|------------------|-------------------------|------------------|-----------------------|----|
|  | Dry<br>weight<br>in mg. | Sporu-<br>lation | Dry<br>weight<br>in mg. | Sporu-<br>lation | Dry<br>weight<br>in mg. | Sporu-<br>lation | Final<br>change<br>pH | of |
| 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                   |                  | $\mathbf{Trace}$        |                  |                       |    |
| Average  | е                       |                  | -                       |                  |                         |                  |                       |    |
| dry<br>weight  | 164,0                   |                  | 163,4                   |                  | 164,6                   |                  |                       |    |

 $\begin{array}{c} {\rm Table~2} \\ {\rm Showing~the~average~dry~weight~and~sporulation~of~three~different~mutants~of} \\ {\it A.~clavatus} \end{array}$ 

| Albino            |  | Tan   |  | Buff   |   |
|-------------------|--|---|--|--|---|
| Dry wt.<br>in mg. | Sporu-<br>lation   | Dry wt.<br>in mg.   | Sporu-<br>lation   | Dry wt.<br>in mg.  | Sporu-<br>lation                                      |
| 122,27            | ++   | 114,5   | +++  | 116,9  | 1/2++   |
| 194,1             | +++  | 208,5   | + + + +  | 182,7  | ++++  |
| 218,4             | ++++   | 204,6   | ++++   | 198,4  | + + + +   |
| 171,9             | ++++   | 136,9   | +++  | 145,4  | + + +   |
| 168,5             | ++   | 147,9   | +++  | 142,3  | +++   |
| 138,8             | ++   | 195,0   | +++  | 161,9  | +++   |
| 119,7             | ++   | 79,2  | ++   | 82,3   | ++  |
| 85,0              | ++   | 96,2  | ++   | 111,6  | +++   |
| No growth         | No growth  | No growth   | No growth  | No growth  | No growth   |
|                   |  |   |  |  | ,   |
| 135,4             |  | 131,4   |  | 126,8  |   |
|                   | Dry wt.<br>in mg.<br>122,27<br>194,1<br>218,4<br>171,9<br>168,5<br>138,8<br>119,7<br>85,0<br>No growth | Dry wt. Sporu- in mg. lation  122,27 ++ 194,1 +++ 218,4 ++++ 171,9 ++++ 188,5 ++ 119,7 ++ 85,0 ++ No growth No growth | Dry wt. in mg.         Sporulation         Dry wt. in mg.           122,27         ++         114,5           194,1         ++++         208,5           218,4         ++++         204,6           171,9         +++++         136,9           168,5         ++         147,9           138,8         ++         195,0           119,7         ++         79,2           85,0         ++         96,2           No growth No growth         No growth | Dry wt. in mg.         Sporulation in mg.         Dry wt. Iation           122,27         ++         114,5         +++           194,1         +++         208,5         ++++           218,4         ++++         204,6         ++++           171,9         ++++         136,9         +++           168,5         ++         147,9         +++           119,7         ++         79,2         +++           119,7         ++         79,2         ++           85,0         ++         96,2         ++           No growth No growth No growth No growth No growth | $\begin{array}{c ccccccccccccccccccccccccccccccccccc$ |

#### 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  $30\,^{\circ}$  C.

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#### References

- Agnihotri, V. P. 1964. Studies on Aspergilli XVI. Effect of pH temperature, and carbon and nitrogen interaction. Mycopathol. et. Mycol. Appl. 24: 305—314.
- Bhatnagar, O. K. 1956. M. Sc. Thesis, Allahabad University, India.
- Chauhan, R. P. S. 1953. M. Sc. Thesis, Allahabad University, India.
- Cochrane, C. W. 1958. Physiology of fungi. John Wiley & Sons, Inc., N. Y. Hawker, L. E. 1957. The physiology of reproduction in fungi, Cambridge University Press. London.
- Johnson, J. W. (1923). Relationships between hydrogen, hydroxyl-ion and salt concentration and the growth of seven soil moulds. Iowa Agri. Exp. Sta. Res. Bull. 76: 308—342.
- Raper, K. B., D. I. Fennell and H. D. Tresner, 1953. The ascosporic stage of Aspergillus citrisporus and related forms. Mycologia 45: 671—697.
- Saksena, R. K. & A. K. Sarbhoy, 1960. Utilization of different carbon compounds by two species of *Cunninghamella*. Proc. Nat. Acad. Sci. India Sec. B., 30: 401—409.
- Thom, C. & M. B. Church. 1926. The Aspergilli. The William and Wilkins Company, Baltimore, U.S.A.
- Thom, C. & K. B. Raper. 1945. A manual of *Aspergilli*. The Williams and Wilkins Company, Baltimore, U.S.A.
- Webb, R. W. 1919. Studies in the physiology of fungi in relation to hydrogen ion concentration. Ann. Mo. Bot. Gard. 6: 201—222.
- Wolf, F. A. & F. T. Wolf, 1947. The fungi. Vol. 1. John Wiley & Sons Inc., N. Y.
- 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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