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# CULTURAL STUDIES WITH *CHAETOMIUM BRASILIENSE* BATISTA AND PONTUAL

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The effect of different media on the growth and reproduction of *chaetomium brasiliense* was studied. It was found that of all the media tried, modified czapek-Dox (Basu *et al* 1955) gave the best result. It was also found that 30°C was effective in inducing favourable growth and Perithecia formation. Moreover P— complete and Fries complete media were quite effective in inducing mycelial growth and P— complete medium was most suitable for Perithecial development.

## INTRODUCTION

In an artificial culture the constituents of the medium greatly influence the growth pattern and development in the fungi. Polymorphism in the genus *Chaetomium* was studied by Beauverie (1900) with varying composition of the medium. Observations on cultural studies were reported by Tsehudy (1937) with a few species of *Chaetomium* in media supplemented with various carbohydrates. Ames however, recommended a relatively lean diet to prevent overgrowth of mycelium and profusion of fruit bodies. Extensive studies on the utilization of carbohydrates, nitrogen compounds and other accessory substances on growth and sporulation of *C. globosum* were done by Buston and Basu (1948), Basu (1951) and Basu and Bose (1958). Comparative studies on the growth physiology of

*C. aureum* in different media supplemented with various carbon and nitrogan sources have been done by Ghora (1971) and Chaudhuri (1973). Basu (1952) had reported that *C. brasiliense* requires aneurin (thiamin) for its normal growth in Czapex-Dox salt solutions. Cultural oxperiments with *C. brasiliense* and a few other ascomycetous fungi on mycelial growth and perithecia formation etc. were recently done by Chaudhuri (Chaudhuri, 1976. 1977).

In view of the cellulose decomposing ability of the members of the genus *Chaetomium* and their varied importance in agriculture, industry and storage problems, atudies with *C. brasiliense* were undertaken with varying cultural conditions in order to select favourable media suitable for the expression of specific characters in relation to its growth and reproduction.

## Material and methods

The strain received as a gift from the Indian Jute Industries Association' Calcutta-700 053, (Basu, 1948).

Stock culture was maintained in malt-dextrose agar having carboxymethyl cellulose. Monosporous isolations were done on P-complete (Westergarrd and Mitchell, 1947) or $A_2$/48 (Chaudhuri, 1964) solid medium from ascospores. The media were autoclaved at 10 lb pressure for 20 minutes the pH being adjusted to 6.5 before sterilization and all inoculations were done with 0.5 ml suspension of ascospores having a concentration of approximately 20 x $10^6$ per ml.

Auxanographic test (Pontecorvo, 1953; Mitra and Chaudhuri, 1966) with various organic nutrients added to Czapek-Dox medium confirm confirmed the earlier finding (Basu *et al*, 1952) that the strain requires thiamine for its natural growth.

Optimum concentration of thiamine required for growth of the strain was determined by adding separately 5.0, 7.5, 10.0, 12.5 and 15.0 $\mu$g of thiamine per ml of liquid $A_1$ (Chaudhuri, 1964) and modified Czapek-Dox (Basu *et al*, 1955),

The cultures were grown in 100ml Erlenmeyer flasks containing 25ml of medium and growth determined after 15 days of incubation at 30°C by comparison of mean dry wt from each set of 5 replicates. As 10 $\mu$g thiamin per ml gave the optimal growth of mycelium all minimal media were supplemented accordingly. The control set was maintained without any supplement.

Minimal media tried for cultural studies on mycelial growth were Czapek-Dox (Czapek, 1902-1903 ; Dox, 1910), modified Czapek-Dox (Basu *et al*, 1955), Czapek-Dox-Clutterbuck (Clutterbuck *et al*. 1932), Czapek-Dox Mclean (Mclean and Cook, 1941), $A_1$ mininal (Chaudhuri, 1964), P-minimal (Westergaard and Mitchell, 1947), Fries minimal (Fries, 1938), Ryan minimal (Ryan, 1950) and Pontecorvo minimal (Pontecorvo, 1952). 25 ml quantity of broth was dispensed in each 100 ml Erlenmeyer flask and 5 replicates were considered for each observation the controls having no thiamine supplement.

The complete media used for the study of growth response were P-complete (Westergaard and Mitchell, 1947), Standard complete (Pontecorvo, 1953), Fries complete (Fries, 1938) and $A_2$/48 complete (Chaudhuri, 1964). The experiment was set up in the same manner as that with the minimal media. Observation was taken every five days upto 30 days of incubation at 30°C.

Dry weight of mycelium was taken after each specified period of growth following the usual technique (Mukherjee and Chaudhuri, 1975) and pH of residual medium observed.

For the study of perithecial development, cultures were grown in different glass vessels on minimal and complete media. 10 ml and 5 ml of solid media were taken in 8 cm diameter Petridishes and 15 x 155 mm culture tubes respectively. In case of 100 ml. capacity Erlenmeyer flasks 15 ml solid and 25 ml liquid broth of each medium were dispensed. Sets of culture vessels were kept at 25° and 30°C and observations on colonial growth and perithecial development were taken 15 days after inoculation.

## RESULTS

*Effect of different minimal media on growth of mycelium*

Different minimal media were tried with the requisite nutritional supplement. It will be seen from Table 1 that modified Czapek-Dox (Basu *et al*, 1955) gives the most satisfactory mycelial growth as expressed in dry wt.

*Effect of complete media on growth of mycelium*

The data in Table 2 will show that growth performance was at its best in P-complete medium. However $A_2$/48 and Fries complete were also found to induce fair growth of mycelium 10 day period of incubation was most effective.

*Effect of different minimal and complete media on growth and perithecia development under varying cultural conditions*

It will be seen from Table 1 that incubation at 30°C was effective in inducing favourable growth and perithecia formation in almost all the culture vessels tried. Except in $A_1$ and modified Czapek-Dox, liquid broth was not found suitable for effective perithecial development. In general culture tubes were found suitable for mycelial growth and perithecial development as well with all the media tried. It is also evident from Table 2 that P-complete and Fries complete media on the whole are quite effective in inducing mycelial growth. However, the former proved most suitable for perithecial development among all the complete media tried.

**Table : 1** *Mycelial growth and perithecia formation in* Chaetomium brasiliense *in minimal media at 25° and 30°C*

| Medium | Incubation °C (15 days) | Culture vessel: Petridish | | Culture tube | | Erlonmeyer flask(100ml) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Solid | | Liquid | | |
| | | Myceliel growth | Perithecia | Growth | Perithecia | Growth | Perithecia | Growth (mg Dry wt) | Perithecia | Altered pH of medium |
| Modified Czapek-Dox | 25 | +++ | +++ | +++ | +++ | ++ | +++ | 310.50 | +++ | 6.4 |
| (Basu *et al* , 1955) | 30 | ++++ | +++ | ++++ | ++++ | +++ | ++++ | 352.50 | ++++ | 6.5 |
| Czapek-Dox | 25 | +++ | ++ | +++ | ++ | +++ | ++ | 212.00 | ++ | 4.8 |
| (Czapek, 1902-3; Dov, 1910) | 30 | ++++ | ++++ | +++ | ++++ | +++ | +++ | 249.50 | +++ | 4.5 |
| Czapek-Dox | 25 | +++ | ++ | +++ | ++ | +++ | ++ | 292.00 | + | 6.5 |
| (Clutterbuck *et al.*, 1932) | 30 | ++++ | +++ | ++++ | ++++ | ++++ | +++ | 326.50 | +++ | 6.8 |
| Czapek-Dox | 25 | ++ | ++ | +++ | ++ | +++ | + | 275.00 | + | 7.2 |
| (Mclean and Cook, 1941) | 30 | +++ | ++++ | ++++ | +++ | +++ | ++ | 313.50 | ++ | 7.5 |
| $A_1$-minimal | 25 | +++ | +++ | +++++ | +++ | +++ | +++ | 300.50 | ++++ | 6.0 |
| (Chaudhuri, 1964) | 30 | ++++ | ++++ | ++++++ | ++++ | ++++ | +++++ | 333.00 | ++++++ | 6.0 |
| P-minimal | 25 | ++ | ++ | ++ | ++ | +++ | + | 118.00 | + | 6.5 |
| (Westergaard and Mitchell, 1947) | 30 | +++ | ++++ | +++ | + | +++ | ++ | 141.00 | + | 6.5 |
| Fries minimal | 25 | ++ | ++ | ++ | ++ | ++ | ++ | 152.00 | + | 6.7 |
| (Fries, 1938) | 30 | +++ | ++ | +++ | +++ | +++ | ++ | 171.25 | + | 6.0 |
| Pontecorvo minimal | 25 | ++ | ++ | ++ | +++ | ++ | ++ | 135.50 | + | 6.2 |
| (Pontecorvo, 1953) | 30 | +++ | ++ | +++ | +++ | +++ | +++ | 168.00 | + | 6.3 |
| Ryan minima | 25 | ++ | ++ | +++ | ++ | ++ | ++ | 115.00 | + | 6.0 |
| (Ryan, 1950) | | ++ | ++ | +++ | +++ | +++ | +++ | 140.25 | + | 6.0 |

**Table** 2. *Mycelial growth and parithecia formation in* Chaetomium brasiliense *in complete Media at 25° and 30°C under different cultural conditioons*

| Medium | Incubation °C (15 days) | Culture vessel | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Petridish | | Culture tube | | Erlenmeyer flask (100 ml) | | | | |
| | | | | | | Solid | | Liquid | | |
| | | Growth | Perithecia | Growth | Perithecia | Growth | Perithecia | Growth | Perithecia | Altered pH of medium |
| P-complete | 25 | ++ | ++++ | +++ | ++++ | +++ | +++ | 162.00 | +++++ | 7,5 |
| (Westergaard and | 30 | +++ | ++++ | ++++ | +++++ | ++++ | +++ | 184.76 | ++++++ | 7.3 |
| Mitchell, 1947) | | | | | | | | | | |
| Fries complete | 25 | +++ | +++ | +++++ | +++ | +++ | ++ | 232.00 | + | 6.5 |
| (Fries, 1938) | 30 | ++++ | ++++ | ++++++ | ++ | +++++ | +++ | 240.00 | ++ | 6.5 |
| Standard complete | 25 | +++ | ++ | +++++ | ++ | ++++ | +++ | 185.50 | + | 6.4 |
| (Pontecorvo, 1952) | 30 | ++++ | +++ | +++++ | + | +++++ | ++++ | 211.20 | + | 6.8 |
| $A_2$/48 complete | 25 | +++ | +++ | ++++ | +++ | ++++ | ++ | 265.00 | + | 6.5 |
| (Chaudhuri, 1964) | 30 | +++++ | +++ | ++++++ | ++ | +++++ | +++ | 282.25 | ++ | 6.7 |

## Discussion

Cultural tests on modified Czapek-Dox (Basu *et al.* 1955) and $A_1$ minimal (Chaudhuri, 1964) revealed that 10.0 $\mu$g thiamine per ml of medium induces profuse mycelial growth as observed in terms of dry wt. Basu, however, reported that the same strain requires for its growth 12.5 $\mu$g aneurin (thiamine) per ml in the modified Czapek-Dox solution. The difference in observations on the quantity of the nutritional requirement may be due to some adaptibility of the strain under continued cultural conditions.

The reason for the preference of modified Czapek-Dox by the strain is obviously due to incorporation of monosaccharide glucose in the system and its utilization in presence of sodium nitrate. However, sucrose is not found as effective as glucose under similar circumstances with Czapek-Dox original and Czapek-Dox-Mclean. Ammonium nitrate also combines well with glucose in $A_1$ minimal but proves quite unsuitable with sucrose in inducing mycelial growth as is found with Ryan minimal (Table 1). Performance of the strain, however was at its best in $A_1$ minimal considering profusion of mycelial growth and abundance of perithecia in the same although with modified Czapek-Dox a higher value in mycelial weight was observed.

Comparative data on mycelial and perithecial development in four different complete media (Table 2) show preference of the strain to P-complete as the medium encourages profuse formation of perithecia with abundance of ascospores.

Mycelial production in the minimal media does not seem to have any significant correlation with the alteration of pH as the cultures grow in the same (Table 1). Czapek-Dox-Mclean showed an alternation of pH at the level of 7.2 to 7.5 and Czapek-Dox original, between 4.5 and 4.8. The rest of the minimal media varied between 6.0 and 6.7. P-complete medium on the other hand

showed the pH level as high as 7.8 at the end of the incubation period (Table 2). The data reveal that the optimal perithecia production is favoured with less vegetative growth when the complete medium turns alkaline.

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