

CHANGES IN CELLULAR CONSTITUENTS AND
MEDIA DURING MORPHOGENESIS
OF
FOMES LIVIDUS KALCHBR.

By

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Biochemical changes in the morphogenesis of *Fomes lividus* Kalchbr. have been evaluated. The gross changes in the amount of carbohydrate and nitrogen in vegetative mycelium and basidiocarp during different phases of development and the relationship of these changes to the composition of the nutrient medium have been traced. The nitrogen yield has been found to be maximum upto the formation of primordia and glucose is stored in. During the differentiation of the primordia glucose has been extensively used up with slight increase in the nitrogen content of the basidiocarp with decrease of the same in the mycelium. During further differentiation of basidiocarp with formation of different tissues there is loss of both carbohydrate and nitrogen content in the medium.

INTRODUCTION

The biochemistry of morphological changes in fungi has been neglected for a long time. Such studies have so far been made generally with some members of Myxomycetes and Phycomycetes (Cantino and Turian, 1959; Ward, 1959; Wright 1963; Nickerson and Bartnicki-Garcia, 1964). It is noteworthy to mention that except Wessels' work (1965) on *Schizophyllum commune* no work has been done on any other member of Hymenomycetales. References are, however, available for the role of environmental factors on the morphogenesis such as light, humidity, temperature, aeration food supply etc. (Hawker, 1957; Cochrane, 1958; Lowag, 1952; Plunket, 1956, 1958; Madelin, 1956; Banerjee and Bakshi, 1945).

In the present investigation, an attempt has been made to measure the change in the amount of carbohydrate and nitrogen content in the mycelium and in the basidiocarp during different phases of development of *Fomes lividus* Kalchbr and the relationship of these changes to the composition of the nutrient medium.

MATERIAL AND METHODS

Fomes lividus Kalchbr. has been collected from Shyambazar logyard, Calcutta, West Bengal, as a growing basidiocarps on Sal Logs (*Shorea robusta* Gärten f).

From these several monosporous cultures have been made and by random pairing dicaryophasic mycelia have been obtained. These dicaryophasic mycelia are maintained as stock cultures on 2% malt-agar medium for further experimental work.

Pyrex Erlenmeyer flasks (1 litre capacity) are used. In each, 100 gms. quartz sand (acid washed) are poured in, closed with non-absorbent cotton plug and sterilized by dry heat at 160°C. for three consecutive days. After sterilization, the sand is flooded with 50 ml. of sterile medium. The flasks are inoculated with the test-fungus and incubated at 30°C. in diffused light.

The basal medium is a modified one of that of Wessels (1965). The composition of the medium is as follows : Glucose, 20 gm. ; L-asparagine, H₂O, 1.5 gm. ; KH₂PO₄ 0.46 gm. ; K₂HPO₄, 1.00 gm. ; MgSO₄, 7H₂O, 0.5 gm. ; FeCl₃, 6H₂O, 60 gm. ; CuSO₄, 5H₂O, 0.2 mg. ; ZnSO₄, 7H₂O, 2.0 mg. ; MnSO₄, 4H₂O, 0.1 mg. ; Ca(NO₃)₂, 4H₂O, 1.2 gm. ; thiamine-HCl, 120 µg/l and double glass distilled water to make 1 l. The medium is sterilized at 110°C. for 30 minutes.

The dicaryophasic mycelia of *Fomes lividus* are at first grown in malt-agar slants. From it the superficial aerial mycelia are scrapped off and introduced into an Erlenmeyer flask (250 ml.) with 25 ml. of the basal liquid medium and incubated for 7 days at 30°C. in diffused light. After 7 days, the mycelia are taken out from the flasks, washed thoroughly in sterile distilled water and fragmented into small pieces aseptically in an Omnimixer at low speed (ca 3000 r.p.m.). The suspension with the fragmented mycelia are kept in N/15 phosphate buffer solution for 24 hours at 30°C. in diffused light in order to encounter the shock inflicted during fragmentation. From this suspension, 1 ml. aliquots are taken and the culture vessels are inoculated. The density of the suspension does not appear to be of any importance in this experiment excepting in that the thick suspension with high density reduced the lag phase of growth in culture vessels.

During experimental period, at given intervals, the culture vessels are stored at freezing temperature.

The mycelial mat are separated from the sand by the addition of 100 ml. of distilled water and shaking gently to disrupt the mycelial content with sand particles. After this the fluid with the mycelial contents are decanted and filtered through a sintered glass funnel (Jena 3 G). The residue was washed with distilled water and the filtrate is made up to 1 litre for estimation of glucose (Nelson, 1944). The basidiocarps are, however, separated from the mycelia and analysed separately. The mycelial mat are peeled off from the sintered glass funnel and homogenized

with adhering sand particles in 96% ethanol with small glass beads in an Omnimixer for 15 minutes. The resulting homogenate is made upto 25 ml. by adding 80% ethanol making the final concentration of ethanol 70-80%.

The estimation of nitrogen is done following Wessels (1965). The ammonia is determined colorimetrically with Nessler's reagent (Snell and Snell, 1954) at 430 m μ .

The estimation of carbohydrate is also done according to Wessels' (1965) method. 1 ml. of alcoholic homogenate is taken in a tube and the alcohol is removed by holding the tube on a boiling water bath for few minutes. It is then placed in an ice-bath. To it 1 ml. of cold 72% H₂SO₄ (v/v) is added and the fungous material is dissolved by stirring it with a glass rod and finally the volume is made up to 50 ml. Total carbohydrate is determined by the anthrone method as modified by Fairbairn (1953). The measurement is taken at maximal absorption 620 m μ .

RESULT

The results obtained during the experimental periods regarding carbohydrate and nitrogen levels during different phases of development of *Fomes lividus* are given in Table 1.

During early development, it has been noted that if the inoculum is thick suspension, the initial lag phase of growth is reduced. The fungal hyphae at first grow very rapidly within the nutrient medium in a submerged manner for about 2 to 3 days. At this stage the growth is rather poor. Following this the aerial hyphae become more prominent. In next 48 to 72 hours, profuse aerial mycelia are formed. At this stage the differentiation starts. After 6 to 7 days of vigorous growth, the mycelia at some point become much condensed and shows light buff tint. Afterwards these points are found to form primordia which ultimately differentiate into resupinate basidiocarps with distinct regular pore mouths. From the initiation of primordia upto the formation of pores it takes almost 72 hours. In the average, 10 to 15 primordia are formed in each flask but only 8 to 11 basidiocarps are formed.

Table 1. *Data (mean) showing the change in total nitrogen and carbohydrate content of mycelium and basidiocarp of Fomes lividus and of medium during developmental period.*

Incubation Period (Days)	Total Nitrogen (mg.)		Total Carbohydrate (mg. of glucose)		Residual Glu- cose (mg.) in the medium
	Mycelium	Basidiocarps	Mycelium	Basidiocarps	
3	0.516		4.0		840
4	1.012		5.2		756
5	2.865		16.6		703
6	4.012		48.5		685
7	4.362		78.4		610
8	3.562		84.6		530
9	2.894	0.816	68.4	4.2	460
10	2.012	1.242	64.4	12.4	400
11	1.865	1.894	63.8	25.6	260
12	1.800	2.464	62.6	46.2	120
13	1.625	3.026	61.4	44.2	68
14	1.416	3.678	60.8	40.6	15
15	1.280	4.112	60.1	38.2	9
16	1.083	3.894	58.2	36.0	4

From the very onset of growth, total nitrogen content increases in the mycelium and just at the initiation of the primordia, this increment is maximum. The nitrogen is, however, not fully utilized by the mycelium from the basal medium. The greater part of the nitrogen is stored in the mycelium and during differentiation of the primordia this nitrogen is transported into them.

In case of carbohydrate, it has been noticed that during rapid differentiation of the primordia glucose in the basal medium is completely utilized. During complete utilization of glucose of the medium, there is slight increase of nitrogen in basidiocarp accompanied by decrease of nitrogen in mycelium.

During active utilization of nitrogen, there is very little absorption of glucose from the medium but when there is increase of carbohydrate level in the mycelium, there is considerable loss of nitrogen from the mycelium at the same time.

DISCUSSION

The results reveal during the experimental periods make it possible to discuss in a general way some of the gross biochemical events that take place during

morphogenesis of *Fomes lividus*. After studying morphogenesis, the entire process can be divided into 1) undifferentiated submerged vegetative growth, 2) differentiating aerial vegetative growth, 3) initiation of primordia, 4) differentiation of primordia, and 5) formation of mature basidiocarps.

In this experiment it has been found that the initiation of primordia take place in the complete nutritive medium and there exists a competition between different primordia to form the mature basidiocarps. This observations also agree with the views of others (Wakefield, 1909 ; Plunket, 1953) in that the exhaustion of the nutritive substrate triggers in initiation of the primordia and as such there is tremendous competition for food among the primordia.

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