

STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI I. FATTY ACIDS AS CARBON SOURCE OF THREE SPECIES OF *FOMES* AND *GANODERMA*

By

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The effect of different fatty acids, as source of carbon, on the growth of *Fomes senex*, *F. durissimuss*, *F. fastuosus*, *Ganoderma lucidum*, *G. colossus*, and *G. applanatum* was evaluated. Glucose-casein hydrolysate medium was used as basal medium. The effect of oleic, stearic, linoleic, palmitic acid, and in combination was studied and it was found that combination had the maximum stimulatory effect. It is assumed that these exogenous fatty acids are probably utilized by "β-oxidation-condensation route" i.e. the oxidation proceeds by the removal of two carbon atoms at a time and with subsequent recombination of the 2-carbon atom.

INTRODUCTION

The fungi are known to utilize a wide range of natural organic compounds for the synthesis of structural and functional compounds and as sources of energy. Some of them are able to utilize chemically complex carbon-containing compounds for growth while others are selective in their requirements. A wide variety of amino acids, organic acids, sugar and their derivative, alcohol and other more complex organic compounds have so far been tested as a sole source of carbon in the medium for growth of fungi (Ainsworth and Sussman, 1965).

Yusef (1953) first reported that oleic acid has a stimulatory effect on growth of *Polyporus schweinitzii*. Robbins and Hervey (1963) studied the effect of different fatty acids on the growth of *Polyporus schweinitzii* and concluded that ferulic acid has a growth promoting activity. Fries and Yusef (1955) reported that *Ophiostoma multiannulatum* required palmitic, stearic and oleic acid for its growth. In the present investigation the role of different fatty acids on growth of six species of basidiomycetes and at the same time their role as carbon source have been evaluated.

MATERIALS AND METHODS

For the present experiment six different species of basidiomycetes, viz., *Fomes senex* Nees. & Mont., *F. fastuosus* Lev., *F. durissimuss* Lloyd, *Ganoderma*

lucidum (Leyass.) Karst., *G. applanatum* (Pers.) Pat., and *G. colossus* (Fr.) Bres. were taken. The cultures were obtained from Dr. S. N. Banerjee, Head of the Department of Botany, University of Calcutta (Banerjee's Collection No. 191-196). These cultures were subcultured at regular intervals and they were maintained as stock cultures.

Glucose-casein hydrolysate medium was used as basal medium. Pyrex Erlenmeyer flasks (250 ml.) were used as culture-vessels. In each of the required number of flasks, 25 ml. of the basal medium was taken. In the present experiment, glucose was not added in the basal medium. Instead of the glucose, oleic acid, stearic acid, linoleic acid, and palmitic acid were added separately and in combination (1 : 1 : 1 : 1 : : oleic : stearic : palmitic : linoleic). All the above acids were added as salts of sodium. One set was kept as control i.e. with glucose as carbon source. They were plugged and sterilized at 15 lb. pressure for 15 mins. The inoculation was done according to the methods of Lindeberg (1944) but modified by Norkrans (1953). After the inoculation all the flask were incubated (stationary) in an incubator at 30°C. in darkness for 20 days. Sufficient flasks were inoculated to provide five replicates for each treatment for 20 days. The length of the incubation period necessary for optimum growth for treatment was pre-determined by running a series of experiments.

Every fourth day, five replicates of each treatment were harvested five times and the contents were filtered through tared filtered paper (Whatman No. 1) using a Buchner funnel. The residual mycelia were washed with distilled water to remove any trace of the medium and dried at 60°C. in an oven for 24 hrs. After drying, they were kept in a cooled vacuum dessicator, cooled, and later weighed in a chemical balance (Sartorius). The quantitative data thus obtained on individual test nutrient were based on the average dry weight of mycelia produced in the test medium of five flasks. In all the experiments, the pH of the medium was maintained as optimum for each fungus. The basal medium was buffered with N/15 phosphate buffer to maintained the optimum pH.

DESIGN OF THE EXPERIMENT

The design of the experiment was factorial one with five replications. Analysis were based on the idea whether there will be any difference in vegetative growth due to difference in the carbon source among the different species of fungi under two different genera. Regarding the yield of the vegetative growth, fungal interactions with different source of carbon at different incubation periods were considered.

RESULTS

The result obtained during the experimental period have been presented in Tables 1-3. The statistical analysis of variance table has been shown in Table 4.

Table 1. Data (mean) showing the effect of different fatty acids on the fungi.

Fungi (F) $\times$ Fatty acids (T)

F	T	G.a.	G.l.	G.c.	F.s.	F.f.	F.d.	Fatty acids means
Control		285.6	305.2	308.1	108.0	114.5	123.6	207.5
Linoleic acid		285.0	303.9	306.2	106.9	112.6	120.3	205.8
Oleic acid		284.3	302.8	304.1	106.0	112.5	121.7	205.2
Stearic acid		280.1	298.3	297.7	106.8	114.2	119.4	202.7
Palmitic acid		279.8	288.1	293.6	106.5	113.5	119.2	200.1
Combination		298.6	299.8	301.6	105.4	112.6	119.6	206.2
Fungus means		285.5	299.7	301.9	106.6	113.2	120.6	

S.E_m for Fungus (F) = ± 0.00003 S.E_m for Fatty acids (T) = ± 0.00003 S.E_m for the body of F $\times$ T table = ± 0.00017

C.D. for F & T at 5% of P = 0.00010

C.D. for F $\times$ T at 5% of P = 0.00059

Table 2. Data (mean) showing the effect of incubation period on the test-fungi.

Fungi (F) $\times$ Incubation period (I)

F	I	G.a.	G.l.	G.c.	F.s.	F.f.	F.d.	I-means
4 days		278.3	280.5	283.2	99.2	104.3	110.5	192.7
8 days		281.9	290.0	291.7	104.0	109.1	116.6	198.9
12 days		285.8	299.0	300.8	106.6	114.6	120.0	204.5
16 days		289.2	309.8	312.2	109.2	117.6	125.7	210.6
20 days		292.4	319.1	321.5	113.9	120.5	130.3	216.3
F-mean		285.5	299.7	301.9	106.6	113.2	120.6	

S.E_m for Incubation period (I) = ± 0.00002 S.E_m for body of F $\times$ I table = ± 0.00014

C.D. for I at 5% of P = 0.00006

C.D. for F $\times$ I at 5% of P = 0.00048

Table 3. Data (mean) showing the interaction of different Fatty acid (T) and incubation periods (I).

I	T × I						I-means
	Control	Linoleic acid	Oleic acid	Stearic acid	Palmitic acid	Combination of Fatty acids	
T							
4 days	196.1	194.4	193.4	191.1	190.0	191.1	192.7
8 days	201.8	199.9	199.4	197.6	195.2	199.4	198.9
12 days	207.2	205.9	205.28	205.5	199.6	206.5	204.5
16 days	213.2	211.8	211.3	208.5	205.6	213.5	210.6
20 days	219.2	217.1	216.8	214.0	210.3	220.7	216.3
T-means	207.5	205.8	205.2	202.7	200.1	206.2	

S. Em. for I×T = ± 0.00014

C.D. for I×T at 5% of P = 0.0004

Table 4. Analysis of variance table showing the effect of different fatty acids on the growth of the test-fungi at different incubation periods.

Source	d.f.	m.s.	F-value
Fungus (F)	5	1.500900	78994.73*
Fatty acids (T)	5	0.003090	162.63*
Incubation period (I)	4	0.015717	827.21*
F×T	25	0.000071	3.73*
F×I	20	0.000652	65.69*
I×T	20	0.000043	2.26*
Error	820	0.000019	
Total	899		

*Significant at 5% level.

From the foregoing tables (1-3) it will be evident that for all the test fungi the combination of all the four acids is the optimum followed by linoleic acid, and oleic acid. Stearic acid and palmitic acid has negligible effect on the growth of the fungi. Glucose has been found to have best effect as carbon source of all the sources supplied as evident from the control treatment.

DISCUSSION

The effect of fatty acids on the nutrition of fungi are numerous. Yusef (1953) stated that oleic acid stimulated the growth of some basidiomycetes. The synthesis of fatty acids by *Ustilago zae* have been reported in presence of isotopic acetic acid (Fahraeus and Lindeberg, 1953). These exogenous

fatty acids are probably utilized by " β -oxidation-condensation route" i.e. the oxidation proceeds by the removal of two carbon atoms at a time and subsequent recombination of the two carbon fragments to form aceto-acetic acid. The key intermediate is a α -keto fatty acid. Evidence of oxidation in fungi can be had from the investigations of Hofman (1962) and Cheldelin (1961). They showed that the fungus can convert a fatty acid to a methyl ketone of one less carbon atom.

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