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STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI : VIII. EFFECT OF DIFFERENT VITAMINS ON VEGETATIVE GROWTH OF SOME SPECIES OF *FOMES* AND *GANODERMA*

BY

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The role of different vitamins on the growth of all the test-fungi has been recorded. The experimental procedure for this investigation has been described. The different source of vitamins used includes thiamine, pyridoxine, biotin, inositol, riboflavin, *p*-aminobenzoic acid and ascorbic acid. It has been found that all the test-fungi are heterotrophic for thiamine and pyridoxine, but they are partially heterotrophic with regard to *p*-aminobenzoic acid, biotin, inositol and ascorbic acid. In case of riboflavin, it has been noted that all the three species of *Ganoderma* are partially heterotrophic to the vitamin while it has no effect on all the species of *Fomes*.

INTRODUCTION

It is known that fungi require minute quantity of vitamins or growth factors for their normal vegetative growth, reproduction and other vital processes. They are either dependent on or independent of exogenous supply of these vitamins. According to Robbins and Kavanagh (1942) the exogenous requirement or deficiency of a fungus for a vitamin may be absolute or conditioned. It may be single or multiple, complete or partial. Fungi, which are able to synthesize the vitamins are called auxoautotrophic while others who are unable to synthesize them are called auxoheterotrophic (Schopfer, 1943). Our knowledge about the vitamin requirements of fungi has been elaborately reviewed by Hawker (1939), Robbins and Kavanagh (1942), Schopfer (1943), Knight (1945), Fries (1943), Mathur *et al.* (1950), Lilly and Barnett (1951), Cochrane (1958), and Fries (1965).

Though considerable amount of work in this line of research has already been done on higher basidiomycetes yet their utilization by most of the species of *Fomes* and *Ganoderma* under consideration has not been worked out in detail. Kogl and Fries (1937) studied the vitamin requirements of *Polyporus adustus*, *P. abietinus*, *Fomes pinicola*, *Trametes cinnabarina*, *T. serialis* and *Lenzites sepiaria*. They concluded that vitamin B_1 was essential for the nutrition of the aforesaid fungi. Gaumann and Jaag (1951) reported that *Polyporus adustus* was able to utilize vitamin B_1. Rennerfelt and Paris (1953) stated that *Fomes annosus* required vitamin B_1 for its vegetative growth. Fries (1938), Robbins (1938), and Robbins and Kavanagh (1942) demonstrated that thiamine hetrotrophy was common in basidiomycetous fungi. Some of these fungi were capable of synthesizing the thiazole half of the molecule and thus required only the pyrimidine half (Schopfer and Blumer, 1938 ; 1940 ; Leonian and Lilly, 1938 ; Lindeberg, 1944 ; Fries, 1943 ; Norkrans, 1950 and Riggenback, 1957) while others were able to synthesize pyrimidine and required thiazole only (Nocker and Reed, 1943). According to Rennerfelt and Paris (1953) aneurin and pyrimidine, and thiazole had a stimulatory effect on vegetative growth of *Fomes annosus* and *Stereum purpureum* and *Schizophyllum commune* respectively. Fries (1950) investigated the vitamin requirements of twenty one species of basidiomycetes and concluded that they absolutely required thiamine for their growth. Lilly and Barnett (1948, 1949) experimented with several species of basidiomycetes and found that five of them were partially deficient for thiamine while several others were deficient for one or more vitamins. Fries (1938) studied the vitamin requirements of seven species of polyporaceae and concluded that they were completely deficient in aneurin and partially deficient in biotin and inositol. Robbins (1958) studied the vitamin requirements of fifty six species of *Polyporus* and seventeen species of *Fomes* and concluded that they were partially or completely heterotrophic for thiamine and a few were found to be heterotrophic for thiamine and biotin. Ward (1964) stated that low temperate basidiomycetes were heterotrophic for thiamine. Banerjee and Nandi (1965) reported that *Lentinus praerijidus* was deficient of thiamine and pyridoxine. *Polyporus taxanus* was found to be deficient of pantothanic acid (Yusef, 1953) while *Poria vallantii* was the only fungus which required riboflavin as external supply (Jennisson *et al.*, 1955). Samajpati and Banerjee (1966) noted that *Fomes lividus* was completely deficient in thiamine and partially deficient in pyridoxine.

MATERIALS AND METHODS

For the present investigation six species of basidiomycetes, viz., *Fomes senex* Nees & Mont., *F. fastuosus* Lev., *F. durissimus* Ldoyd, *Ganoderma applanatum* (Pers.) Pat., *G. lucidum* (Layss.) Karst., and *G. colossus* (Fr.) Bres. have been collected from Calcutta and suburbs. Tissue cultures have been made from each of the basidiocarps and these tissue cultures were used in the experiment.

Glucose-casein hydrolysate medium (Leonian and Lilly 1945) has been selected as standard basal medium for the present investigation as it has been recommended as a well balanced medium by Lilly and Barnett (1949). The other necessary experimental procedures were same as described in Samajpati and Banerjee (1966).

The standard basal medium was first prepared but without any vitamin source. After preparation, the medium was treated with activated charcoal powder (5g/litre), boiled for five minutes and finally filtered. The charcoal treatment was done in order to avoid any trace of vitamins that may be present in the medium. Further this experiment was carried out according to the method of Burkholder

(1943) for deficiency studies on vitamins. The deficiency of a specific vitamin was detected against a test fungus by its inability to grow in a medium which contained all the vitamins to be tested excepting that specific one. The vitamins were added to the medium as 5 μg/1. of riboflavin, biotin, inositol, and 100 μg/1. of thiamine, pyridoxine, ascorbic acid and *p*-aminobenzoic acid. The media were then sterilized by filtration as stated before (Samajpati and Banerjee, 1966) inoculated seperately by the test-fungi and incubated in the usual way. Several flasks with the medium but without vitamins were also inoculated as controls. The other necessary experimental procedures were, however, remained the same as in Samajpati and Banerjee (1966).

RESULTS

The results obtained during the experimental periods are given in Tables 1—3.

Table 1. *Data (mean) showing the effect of different vitamins on the vegetative growth (mg.) of three species of* Ganoderma *and* Fomes *at different incubation periods*

Fungi (F) × Vitamins (T) table

Fungi / Vitamins	G. applanatum	G. lucidum	G. colossus	F. senex	F. fastuosus	F. durissimus	T- means
Riboflavin	313.0	306.8	303.0	107.4	113.6	122.5	211.1
Biotin	313.5	302.9	305.2	109.1	115.9	124.9	212.0
Inositol	320.2	305.8	306,5	111.4	117.2	128.3	214.9
Ascorbic acid	326.1	313.2	317.7	138.6	132.6	144.6	228.8
p-aminobenzoic acid	341.5	315.5	322.7	142.9	143.8	149.0	235.9
Pyridoxine	345.8	317.1	323.9	144.5	145.7	152.4	238.2
Thiamine	353.8	318.8	325.3	146.2	147.5	154.2	254.3
Control	298.6	299.8	301.7	105.6	112.6	119.7	206.3
F-means	326.6	310.0	313.3	125.7	178.6	137.0	

S. E. for F = ± 0.000264 C. D. at 5% of P = 0.007341
S. E. for T = $+0$ 0024 C. D. at 5% of P = 0.006674
S. E. for F × T = ± 0.00580 C. D. at 5% of P = 0.016213

Table 2. *Data (mean) showing the effect of incubation periods on the vegetative growth (mg.) of three species of* Ganoderma *and* Fomes *under different vitamins sources*

Fungi (F) × Incubation periods (I)

Fungi / Incubation periods (days)	G. applanatum	G. lucidum	G. colossus	F. senex	F. fastuosus	F. durissimus	I-means
4	298.0	288.9	292.4	111.6	113.5	121.4	204.3
8	312.3	299 4	303.4	117 4	122.3	129.9	214.1
12	326.3	309.6	312:9	124.3	130-0	137.1	223.3
16	341 0	321.0	323.1	133.3	135.5	151.8	233.1
20	355.1	330,9	334.7	141.9	141.7	151.8	242.7
F-means	326.5	310.0	313.2	125.7	128.6	136.9	

S. E. for I = ± 0.00187 C. D. at 5% of P = 0.005200
S. E. for T × I = ± 0.00458 C. D. at 5% of P = 0.012736

Table 3. *Data (mean) showing the effect of incubation periods and different vitamins on the growth (mg.) of three species of* Fomes *and* Ganoderma

Incubation periods (I) × Vitamins (T)

Incubation periods (Days) Vitamins	4	8	12	16	20	T-means
Riboflavin	193.6	203.2	212.7	219.0	226.9	211.1
Biotin	195.5	204.0	212.1	219.4	228.5	212.0
Inositol	197.5	206.3	215.7	223.7	231.7	214.9
Ascorbic acid	208.6	218.1	228.0	238.2	250.9	228.8
p-aminobenzoic acid	213.7	225.0	234.5	248.1	258.0	235.9
Pyridoxine	215.7	227.3	237.0	250.6	260.3	238.2
Thiamine	218.2	229.3	240.4	252.3	264.5	254.3
Control	191.3	199.4	206.5	213.5	220.7	206.3
I-means	204.3	214.1	223.3	233.1	242.7	

S. E. for $I \times T = \pm 0.00520$ C. D. for $1 \times T$ at 5% of $P = 0.014461$

From the foregoing Tables 1—3, it is evident that all the six test-fungi are heterotrophic with regard to thiamine and pyridoxine. The stimulatory effect of para-aminobenzoic acid on growth of all the fungi also reveals their heterotrophic nature in the specific vitamin. Biotin, inositol and ascorbic acid have favourable effects on all the test-fungi. Riboflavin has but slight stimulatory effect on growth of all the three species of *Ganoderma*, while it has almost no effect on all the species of *Fomes*. Therefore, it can be stated that all the test-fungi are heterotrophic for thiamine and pyridoxine but they are partially heterotrophic with regard to *p*-aminobenzoic acid, biotin, inositol and ascorbic acid.

DISCUSSIONS

After studying the role of so[illegible] vitamins on the nutrition of all the test-fungi, it has been noticed that all of them are heterotrophic with regard to thamine and pyridoxine (Table 1—3). The stimulatory effect of *p*-aminobenzoic acid on growth of all the fungi also reveals their partial heterotrophic nature with regard to this vitamin. Biotin, inosital, and ascorbic acid have also favourable effects while riboflavin has stimulatory effect only on the three species of *Ganoderma* under study.

The above findings can also be explained with the help of present idea on vitamin metabolism of fungi.

The test-fungi probably utilize thiamine and pyridoxine directly by absorption and storing greater portion of them within the mycelium. During utilization of thiamine fungi may break down the thiamine into its two moieties, pyrimidine and thiazole. The maximum stimulatory effect of this vitamin probably proves its vital role in carbohydrate metabolism and its active part in the formation of thiamine pyrophosphate. In case of pyridoxine, the active role is in the formation of pyridoxal phosphate, which function as coenzyme in the transformation of tryptophane to indole compound (Wood *et al*, 1947). The effect of pyridoxine on growth depends on the presence of thiamine has been noted in the present study, but the real interaction mechanism is still unknown. Para-aminobenzoic acid is also directly utilized by the test-fungi and the vitamin is probably used as precursor of acid synthesis. Its role in triosynthesis of amino acids and previous to some extent has been questioned recently (Cutts and Rainbow, 1950 ; Nyman and Fries, 1962). In all probability biotin, inositol and ascorbic acid are also utilized directly by all the fungi. It is very difficult to find out the partial or total heterotrophy in case of biotin due to its very slow rather low growth rate. It is important for fungal metabolism due to its role in the conversion of ornithine to citruline, in carboxylase reaction, in the hexokinase system, in the synthesis of fatty acids and in the deamination of certain amino acids (Fries, 1965). It is also very difficult to understand the interaction mechanism between biotin and inositol (Tirunarayanan and Sarma, 1953). The role of inositol and ascorbic acid in the metabolism is still speculative and it has been tentatively concluded that inositol is necessary for maintaining the structure of essential cytoplasmic structure, possibly the mitochondria (Ridgeway and Douglas, 1958). The slight stimulatory effect of riboflavin in case of *Ganoderma* is probaby due to its role to provide the prosthatic group of a number of oxidizing enzymes and in the synthesis of nicotinic acid or its role in photoreceptor mechanism as riboflavin protein complex (Fries, 1965).

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