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STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI : XII. AMINO ACIDS AS CARBON SOURCES OF *GANODERMA APPLANATUM* (PERS.) PAT.

By

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Almost all the amino acids tested have supported the vegetative growth of *Ganoderma applanatum* as sole sources of carbon and nitrogen. Of these, aspartic acid and glutamic acid have maximum effect, followed by asparagine, glycine, histidine, proline and alanine. Lysine, citruline, and arginine have but modest effect on growth while tyrosine and serine have almost negligible effect. Presumably, glutamic acid is utilized by the coenzyme I-linked glutamic dehydrogenase, which has been found in the cell free extracts of *Ganoderma applanatum*, via the TCA cycle. In case of aspartic acid, aspartic dehydrogenase and aspartase have also been noticed. Possibly through this path, the members of the series enter the TCA cycle.

INTRODUCTION

Amino acids have been found to support good growth for some hymenomycetous fungi (Chahal, 1967 ; Da Santos, 1963 ; Findlay, 1934 ; Fries, 1954 ; Jennison *et. al.* 1955, and Leonian and Lilly, 1938). There has been almost no work on the use of amino acids as source of carbon by the members of hymenomycetes. It has been found that asparagine and glycine are better sources as amino acids for growth of *Ganoderma applanatum* (Pers.) Pat. (Samajpati, 1969). The present investigation was undertaken to find out whether these amino acids can be utilized as carbon sources by *G. applanatum* and also to elucidate the enzymatic basis of the specific responses exhibited by the test-fungus towards amino acids as carbon sources.

Materials and Methods

Test-fungus. The test-fungus *Ganoderma applanatum* (Pers.) Pat., was obtained from the collection of Dr. S. N. Banerjee of the Department of Botany, University of Calcutta, India. It was subcultured at regular intervals on *potato dextrose agar* medium slants and maintained as stock-culture for further studies.

Culture medium. The *glucose casein hydrolysate* inmedium (Leonian & Lilly, 1945) has been selected as basal synthetic medium for growth of the fungus, as it has been recommended as a well balanced medium (Lilly and Barne, 1949). This medium has been used but without any carbon and nitrogen source as such the medium has the following composition : KH_2PO_4, 1.0 gm ; $MgSO_4$, $7H_2O$, 0.02 gm ; $MnSO_4 7H_2O$, 0.01 gm ; Na_2CO_3, 1.12 gm ; $FeSO_4$, $7H_2O$, 0.02 gm ; $ZnSO_4$, $7H_2O$, 0.02 gm ; and distilled water to make 1000 ml. This medium was distributed in 30 ml amounts in 250 ml Erlenmeyer flasks which were used as culture vessel and was sterilized by autoclaving at 15 lbs pressure for 15 minutes. All additions to this medium were neutralized, sterilized separately and added aseptically.

Inoculation and Incubation. The inoculation method employed in this investigation was that of Lindeberg (1944) but with modification. A double-layered, agar medium, of which 20 ml cleared and purified agar was allowed to solidify before the upper layer of 15 ml was added, was prepared on a Petridish (100 cm.) and inoculated with the fungus. The inoculum discs (5 mm diam.) were punched out aseptically by a stainless steel cutter from the advancing zone of the mycelium and they were then placed on a new agar plate and incubated for 24 hrs. at 30°C in diffuse light. This was necessary to overcome the shock encountered by the fungal mycelia during punching. Each culture vessel was then inoculated with one inoculum disc with the mycelial side upwards The purified and cleared agar was treated with activated charcoal powder (5 mg/litre) for removing any growth promoting substances from the agar.

After inoculation, the culture vessels were incubated at 30°C on a shaking incubator in diffuse light which was set at 250 r p m. Sufficient culture-vessels were incubated to provide five replicates of each treatment for 20 days. The length of the incubation period necessary for optimum growth for each treatment was predetermined by running a series of experiments. The *p*H of the medium was maintained at 6.0 with N/15 phosphate buffer.

Measurement of Growth. After the incubation period, five replicates of each treatment were harvested and the mycelial contents were filtered through a tared Whatman No. 1 filter paper. The residual mycelia were washed with distilled

water to remove any trace of the medium. The mycelia along with the tared filter paper were then dried at 60°C for 24 hrs and weighed. The taring of the filter paper was done under the same condition. The difference in weight was taken as mycelial dry weight.

All the chemicals used were of analytical grade and were obtained from commercial sources.

Cell-free enzyme preparation. The cell-free extract preparations used in the transaminase and dehydrogenase studies were prepared in the following way : To the basal medium, sodium glutamate and sodium aspartate (0.5 per cent) and glucose (1.0 per cent) were added, in which the fungal mycelia were grown. The culture-vessels were incubated for 72 hrs at 30°C on the shaking incubator. The cells were harvested by centrifugation, and were washed twice with distilled water and twice with M/40 phosphate buffer. During washing the liquid was squeezed out from the mycelia through muslin to remove adhering substrate. The *p*H 7.2 was maintained in the transaminase studies and *p*H 7.0 in the dehydrogenase studies. The mycelia were next macerated in an Omnimixer for about 45 seconds at high speed in excess buffer. The resulting mycelial fragments were again washed with distilled water and twice with buffers and then resuspended in buffer solution containing approximately 25 mg cells per 5 ml. This cell suspension was subjected to disintregation in an Omnimixer at 4°C at 5000 rpm to break the individual cells. The suspension was then centrifuged at 30,000×G for 20 minutes in the cold to remove whole cells, the supernatant was used for enzyme assays.

Enzymatic studies. Transaminase activity was determined according to the method of Romano and Nickerson (1958) Dehydrogenase activity was determined by the method of Fahmy and Walsh (1952). Optical density readings were taken on a Klett-Summerson Photoelectric Colorimeter.

RESULTS

Growth with amino acids as sole sources of carbon and nitrogen.

As shown in table 1, aspartic acid, glutamic acid asparagine, glycine, proline and histidine support relatively good growth followed by modest growth by lysine, arginine, alanine, tyrosine, trytophane, while serine permit very slight growth of the test-fungus.

Table 1 *Vegetative growth of* Ganoderma applanatuu *with single amino acid as sole source of carbon and nitrogen.*

Addition to Basal Medium*	Growth** mg dry wt/30 ml culture	$S\bar{x}$
L-Glutamate, monosodium	113	0.14
L-Aspartate, momosodium	114	0.20
L-Asparagine	96	0.12
Glycine	99	0.12
L-Proline	85	0.16
L-Histidine-HCl	87	0.14
L-Lysine-HCl	58	0.16
Citruline	59	0.12
DL-Alanine	78	0.12
DL-Tyrosine	32	0.32
L-Arginine-HCl	44	0.30
DL-Serine	34	0.28
Control***	29	0.14

* All substances were sterilized separately in aqueous solutions, and added aseptically to give final concentrations of 2 g per 100 ml medium.

** Culture incubated with continuous shaking for 20 days and all the results are mean of five replicates.

*** Basal medium without any source of nitrogen.

$S\bar{x}$ =Standard deviation of the means, calculated by the formula, $S\bar{x} = \frac{(x-x)2}{n(n-1)}$

Dehydrogenase studies. It has been tested to see the enzymatic pathways of aspartate and glutamate metabolism as these two compounds gave best effect on growth. The utilization of aspartate and glutamate as an energy source would presumably take place through the agency of aspartic dehydrogenase and glutamic dehydrogenase, which actually catalyze the oxidative deamination of aspartate and glutamate. Further oxidation procedure would follow via the tricarboxylic acid cycle.

It has been found that there is a rapid reduction of 2, 3, 5-triphenyltetrazolum chloride (TTC) in the presence of the crude enzyme preparation, coenzyme 1 (DPN), and sodium aspartate and sodium glutamate (Fig. 1). This confirms the presence of an aspartic and glutamic dehydrogenase.

Transamination studies. In the presence of dialyzed cell-free extracts, pyridoxial phosphate, glutamate and oxaloacetate, the formation of aspartate was detected and in the presence of cell-free extracts, pyridoxal phosphate, aspartate, and ∝-ketglutarate, the formation of glutamate occurred. It is thus clear that operation of TCA cycle in the aspartate and glutamate metabolisms performed in presence of available amino acceptor.

Discussion

The present study on utilization of various amino acids as sole sources of carbon and nitrogen by *Ganoderma applanatum* have revealed some interesting points in the metabolism of this organism specifically and in the metabolism of the basidiomycetous fungi in general. The amino acids belonging to the primary series, i.e. glutamic acid series (Samajpati, 1969) in utilized as source of carbon. The members of secondary series, aspartic acid, asparagine, alanine, lysine, histidine, proline, glycine, tyrosine, and serine, supported the growth of the test-fungus as carbon sources. The tertiary members, citruline, and quaternary members, arginine, have also supported the growth of the test-fungus but the effect is negligible in comparison to others.

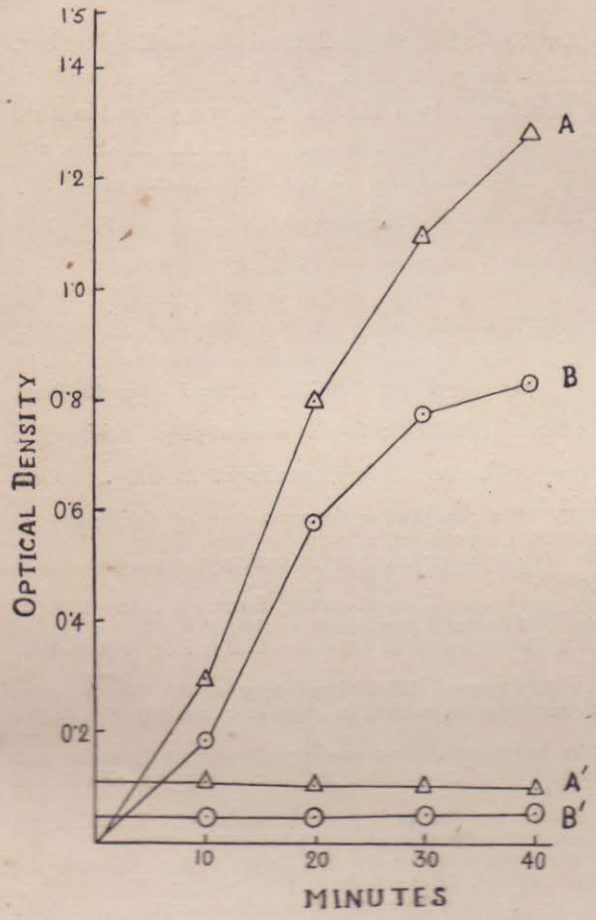

Fig. 1. Graph showing the rate of reduction of 2, 3, 5-TTC by cell free extract of *Ganoderma applanatum* in the presence of
A —Extract + DPN +Sodium glutamate ;
A′—Extract+DPN, Extract +DPN+ Sodium aspartate ;
B —Extract+TPN +Sodium glutamate ; and
B′—Extract+TPN, Extract+TPN+ Sodium aspartate.

From the available data, it can be assumed that these amino acids are probably utilized by *Ganoderma applanatum* as sources of energy through the tricarboxylic acid pathway. The extensive operation of the TCA cycle in the higher fungi has been already established. It has also been proved that on the way of catabolism of carbon compounds by the EMP pathway to the TCA cycle, the C-fragments become incorporated into amino acids somewhere on the route (Burnett, 1968 ; Nicholas, 1965). It can also be assumed that aspartic acid

and glutamic acid are linked with TCA cycle in presence of an ammonium acceptor (possibly ∝-ketoglutarate) as it has been found that active transamination is present or the aspartic acid is converted to fumarate in the presence of aspartase, resulting two direct pathways between the amino acids and TCA cycle.

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REFERENCES

Burnett, J. H. 1968. Fundamentals of Mycology. pp. 286-296. Edward Arnold Press (Publishers) Ltd., London.

Chahal, D. S. 1967. The growth of selected cellulolytic fungi on wood pulp. *Diss. Abst.*, **27** (9) B : 2997-2998.

Da Santes, A. N. F. D. A. 1963. Nitrogen utilization by four isolates of *Armellaria mellea*. Trans. Brit. mycol. Soc. **46** (2) : 281-286.

Fahmy, A. R , and E. O'F. Walsh. 1952. The quantitative determination of dehydrogenase activity in cell suspensions. Biochem. J. (London). **51** : 55-56.

Feldman, L. I., and I. C. Gunsalus. 1950. The occurrence of a wide variety of transaminases in bacteria. J. Biol. Chem. **187** : 821-830.

Findlay, W. P. K. 1934. Studies in the physiology of wood-rotting fungi. I. The effect of nitrogen content upon the rate of decay of timber. Ann. Botany. **48** : 109-117.

Fries, N. 1954. The responses of some basidiomycetes to constituents of nucleic acid. Svensk. bot. Tidskr. **48** (2) : 559-578.

Jennison, W. M., M. D. Newcomb and R. Henderson. 1955. Physiology of the Wood-rotting Basidiomycetes. I. Growth and nutrition in culture in synthetic media. Mycologia. **47** : 275-304.

Lilly, V. G., and H. L. Barnett. 1949. The influence of concentration of nutrients thiamine, and biotine upon growth and the formation of perithecia and ascospores by *Chætomium convolutum*. Mycologia. **48** : 186-196.

Leonian, L. H., and V. G. Lilly. 1938. Studies on the nutrition of fungi. VI. Thiamine, its constituents and the source of nitrogen. Phytopathology. **28** ; 531-548.

————, and ————. 1945. The comparative value of different test organisms in the microbiological assaya of B-vitamins. West. Va. Agric. Expt. Sta. Bull., pp. 319.

Lindeberg, G. 1944. Uber die Physiologie Ligninabbauender Bodenhymenozyten. Symbolæ Botan. Upsaliensis. **8** (2) : 1-183.

Nicholas, D. J. D. 1965. Utilization of inorganic nitrogen compounds and amino acids by fungi. In *The Fungi*, Vol. I. : 349-376. Edited by G. C. Ainsworth and A. Sussman. Academic Press, New York.

Romano, A. H., and W. J. Nickerson. 1958. Utilization of amino acids as carbon source by Streptomyces fradiæ. Jour. Bacteriol. **75** (2) : 161-166.

Samajpati N. 1969. Physiological studies on some species of *Fomes* and *Ganoderma*. D. Phil. Thesis, University of Calcutta.

Sims, A. P., and B. F. Folkes. 1964 A kinetic study of the assimilation of ^{15}N-ammonia and the synthesis of amino acids in an exponentially growing culture of *Candida utilis*. Proc. Roy. Soc., B. **159** : 479-502.

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