

EFFECT OF DIFFERENT SOURCES OF NITROGEN ON  
THE GROWTH OF SOME SPECIES OF  
*POLYPORUS* AND *FOMES*

By

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The present investigation has been carried out to provide information about the role of different sources of nitrogen as a nutritional factor on the vegetative growth of *Polyporus cinnabarinus* Jacq. ex. Fries., *Fomes lividus* Kalchbr., and *Polyporus zonalis* Berk. Experimental procedures for this investigation have been discussed. Of the different sources of nitrogen used, DL-alanine, L-proline, L-arginine, glycine and casein hydrolysate have been found to be good sources for *P. cinnabarinus*, while in cases of *F. lividus* and *P. zonalis*, these are L-asparagine, DL-alanine, L-arginine and casein hydrolysate and L-asparagine, glycine, L-proline, L-arginine and casein hydrolysate respectively.

INTRODUCTION

The growth responses of fungi to nitrogen from amino-acids are very diverse as they vary with the organisms used and with factors of environment. As yet, no general correlation between the taxonomic position of fungi and their growth responses to amino-acids have been made. Even biotypes of one organism differ greatly in their amino-acid nutrition. However, in general, fungi do not have natural absolute requirement for a particular amino-acid (Foster, 1949). Generalizations from studies on amino-acids in relation to fungus cannot be easily made, partly because of the dependence of results on diverse experimental conditions. Different amino-acids often induce different growth responses.

Although some work has so far been done on the nutritional requirements of amino-acids nitrogen by basidiomycetes, its utilization by fungi under investigation has not been worked out yet. Findlay (1934) stated that the organic sources of nitrogen stimulated the growth of *Trametes seriales* and *Polystictus versicolor*. Leonian and Lilly (1938) found that *Coprinus lagopus* and *Phenrotus corticatus* could grow well on a mixture of five amino-acids as source of nitrogen. Fries (1950, 1954) studied extensively the nitrogen requirements of several basidiomycetes and concluded that the nitrogen requirements of these fungi may be autotrophic or heterotrophic. Lilly and Barnett (1951), associated the 2 stereoisomers, L and D, of amino-acids with different growth responses. Banerjee and Nandi (1965) stated that the best source of nitrogen for the vegetative growth

of *Lentinus praerigidus* was glycine. Samajpati (1972) found asparagine and ammonium sulphate as the best organic and inorganic sources of nitrogen for the growth of a number of species of *Ganoderma* and *Fomes*.

The present studies on the growth responses of both the types of mycelia of the test-fungi to a number of amino-acids used as sources of nitrogen have been taken up.

#### MATERIALS AND METHODS

For the present investigation three species of Basidiomycetes viz. *Polyporus cinnabarinus* Jacq. ex. Fr., *Polyporus zonalis* Berk. and *Fomes lividus* Kalchbr. were collected from Calcutta and suburbs. Monokaryophasic, dikaryophasic and polyporus cultures were made from the fresh basidiocarps of the test-fungi following usual procedures and these cultures were used in the experiment.

The basal liquid medium used during these studies was *glucose-casein-hydrolysate* medium (Leonian and Lilly, 1945). It was prepared without any nitrogen source. The sources of nitrogen were added to the basal medium in amounts calculated to give equivalent concentrations of nitrogen. Three concentrations were used: 0.05, 0.1 and 0.2 gm. nitrogen/litre. The sources used were glycine, DL-alanine, L-asparagine, DL-isoleucine, L-histidine, DL-tryptophane, L-proline, L-arginine and casein hydrolysate. The media were then sterilized, inoculated separately by the test-fungi and incubated stationary. Several flasks with basal medium without any nitrogen source were also kept as controls. There were three incubation periods of 8, 12 and 16 days, and each treatment had three replicates.

The other experimental procedures were, however, remained the same as described by Samajpati (1972).

#### RESULTS

The results obtained are given in Tables 1-3.

Table 1. Data (mean) showing the effect of incubation period (days) on the vegetative growth (mg.) of the test-fungi in presence of different sources of nitrogen.

Incubation period (Days)	Fungi (F) × Incubation period (I).							Incubation period means.
	<i>P. cinnabarinus</i>		<i>F. lividus</i>		<i>P. zonalis</i>			
Fungi	Pr. mycelia	Sec. mycelia	Pr. mycelia	Sec. mycelia	Pr. mycelia	Polys. mycelia		
8	34.3	53.6	101.3	110.8	150.0	122.4	95.4	
12	58.7	71.7	125.1	138.3	171.4	147.0	118.7	
16	117.3	145.4	207.3	216.3	258.6	223.0	194.4	
Fungi mean.	70.1	90.3	144.6	155.2	193.4	164.2		

S.E. for F mean = ±0.000731  
 S.E. for I mean = ±0.000661  
 S.E. for F × I mean = ±0.00151

C.D. for F at 5% of P = 0.002042  
 C.D. for I at 5% of P = 0.001741  
 C.D. for F × I at 5% of P = 0.004162

Table 2. Data (mean) showing the effect of nitrogen sources on the vegetative growth (mg.) of the test-fungi at different incubation periods.

Nitrogen sources (T) × Fungi (F).

Nitrogen sources	Conc. of Nitrogen	<i>P. cinnabarinus</i>		<i>F. lividus</i>		<i>P. zonalis</i>		T-means.
		Pr. mycelia	Sec. mycelia	Pr. mycelia	Sec. mycelia	Pr. mycelia	Polys. mycelia	
L-Asparagine	0.05	51.0	76.3	199.2	218.0	308.0	295.0	191.2
	0.10	53.9	84.2	193.1	217.0	320.0	220.0	181.3
	0.20	51.2	78.0	191.6	212.0	311.0	231.0	179.1
DL-Alanine	0.05	77.2	99.0	206.0	221.0	102.0	90.2	132.5
	0.10	81.6	112.0	210.1	221.0	98.0	90.5	135.5
	0.20	80.2	116.0	212.0	223.0	92.0	97.0	136.7
Glycine	0.05	79.2	102.0	146.0	154.0	281.0	258.0	170.0
	0.10	88.6	112.0	146.0	154.2	275.6	252.0	171.4
	0.20	87.2	108.0	150.1	157.0	275.0	249.0	171.0
DL-Isoleucine	0.05	52.8	74.0	60.2	68.0	120.0	110.0	80.8
	0.10	70.1	95.0	64.1	80.0	132.0	108.0	91.5
	0.20	66.2	93.0	68.2	70.0	130.0	109.0	89.4
L-Histidine	0.05	34.2	46.0	99.2	110.0	70.0	60.0	69.9
	0.10	43.6	48.2	102.0	110.5	66.0	58.0	71.4
	0.20	41.2	45.2	108.6	114.6	61.0	59.0	71.6
DL-Tryptophane	0.05	42.2	43.0	57.2	61.0	60.0	60.0	53.9
	0.10	46.0	40.1	52.6	63.0	65.0	50.0	52.7
	0.20	42.2	34.2	53.1	60.0	61.0	51.0	50.2
L-Proline	0.05	80.1	108.0	110.2	110.0	207.0	190.0	134.2
	0.10	93.2	116.0	102.1	126.0	205.0	189.0	138.5
	0.20	88.2	100.7	112.0	128.0	203.0	182.0	135.6
L-Arginine	0.05	90.2	116.0	137.0	140.0	260.0	231.0	162.3
	0.10	114.6	138.0	142.0	158.0	275.0	220.0	174.4
	0.20	92.0	130.0	151.0	151.0	261.0	227.0	168.6
Casein hydrolysate	0.05	98.4	121.0	318.0	328.6	381.0	300.0	257.8
	0.10	111.2	142.0	320.0	332.0	385.0	288.0	263.5
	0.20	102.6	141.0	330.0	340.0	382.0	300.0	265.9
Control		4.6	9.6	8.6	18.6	27.0	25.0	15.5
Fungi means		70.1	90.3	144.6	155.2	193.4	164.2	

S.E. for T = ±0.0021

S.E. for T × F = ±0.00241

C.D. for T at 5% of P = 0.002678

C.D. for T × F at 5% of P = 0.00681

Table 3. Data (mean) showing the effect of incubation periods (days) on the role of nitrogen sources on the vegetative growth (mg.) of the test-fungi.

Nitrogen source (T) × Incubation period (I)		Incubation period			T means
Nitrogen sources	Conc. of Nitrogen	8 days	12 days	16 days	
L-Asparagine	0.05	130.4	180.6	262.6	191.2
	0.10	128.5	169.8	245.6	181.3
	0.20	123.5	167.2	246.6	179.1
DL-Alanine	0.05	106.0	128.5	163.0	132.5
	0.10	106.5	129.0	162.0	135.5
	0.20	111.1	131.0	168.0	136.7
Glycine	0.05	121.0	151.5	237.5	170.0
	0.10	120.5	152.0	241.7	171.4
	0.20	119.0	151.0	243.0	171.0
DL-Isoleucine	0.05	37.2	57.8	147.4	80.8
	0.10	42.0	61.6	170.9	91.5
	0.20	41.0	60.2	167.0	89.4
L-Histidine	0.05	34.0	51.0	124.7	69.9
	0.10	35.2	54.0	125.0	71.4
	0.20	35.0	53.5	126.3	71.6
DL-Tryptophane	0.05	26.3	40.1	95.3	53.9
	0.10	26.5	40.0	91.6	52.7
	0.20	26.4	40.2	84.0	50.2
L-Proline	0.05	94.6	114.2	193.8	134.2
	0.10	97.0	116.2	202.3	138.5
	0.20	96.7	115.0	195.1	135.6
L-Arginine	0.05	120.0	141.0	225.3	162.3
	0.10	129.2	151.0	243.0	174.4
	0.20	123.1	147.6	235.1	168.6
Casein hydrolysate	0.05	210.1	231.0	332.3	257.8
	0.10	211.6	237.0	341.9	263.5
	0.20	212.0	239.0	346.7	265.9
Control		7.6	12.6	26.3	15.5
I-means		95.4	118.7	194.4	

S.E. for T × I = ±0.0021

C.D. for T × I at 5% of P = 0.005931

It is evident from the foregoing Tables that there is a great diversity in growth responses induced by different amino acids. These responses are affected by the amino acid concentration and the duration of incubation period. The results also

reveal that casein hydrolysate is the best source of nitrogen for all the test-fungi. It is, nevertheless, possible to classify the amino acids, used in the present studies, into three groups 'good', 'fair' and 'poor', according to whether they usually induce high, intermediate or low mycelial growth respectively. In the case of *P. cinnabarinus* 'good' sources are DL-alanine, L-proline, glycine, and casein hydrolysate, 'fair' sources are L-asparagine and DL-isoleucine, while 'poor' sources are DL-tryptophane and L-histidine. In the case of *Fomes lividus*, 'good' sources are L-asparagine, DL-alanine, L-arginine and casein hydrolysate, 'fair' sources are glycine, L-histidine and L-proline, while the 'poor' sources are DL-isoleucine and DL-tryptophane. For *P. zonalis*, on the other hand, the 'good' sources are L-asparagine, glycine, L-proline, L-arginine and casein hydrolysate, 'fair' sources are DL-alanine and DL-isoleucine, while the 'poor' sources are L-histidine and DL-tryptophane.

#### DISCUSSION

It is evident from the present study on the role of amino acids as nitrogen source on the growth of the fungi under consideration that almost all the amino acids tested are utilized by them. On the average, there is some correlation between the available nitrogen from the amino acid and the amount of growth produced due to it, irrespective of its isomerism. This finding possibly indicates that the growth response due to any specific amino acid is controlled by the carbon chain and its position in respect to amino group (Leonian and Lilly, 1938; and Lilly and Barnett, 1951). Of the aliphatic monoamino monocarboxylic acids containing no additional functional groups, with shorter (3-4) chain length, viz., glycine and alanine, have significantly more growth than the 5- or 6- carbon acid, isoleucine. The monoamino dicarboxylic acids and their corresponding amide (diamino), asparagine, are equally as good as glycine and alanine. The diamino monocarboxylic acid the 5-carbon-chain, arginine is utilized to the same extent as glycine. The heterocyclic tryptophane, histidine and proline fit into no particular pattern. Proline is good nitrogen source and histidine a poor one with the other intermediate in growth supporting ability. Casein hydrolysate is better than any single amino acid for all the three organisms. Of the individual organic nitrogen compound tested asparagine, glycine, alanine, arginine and proline have the maximum stimulatory effect on all the test-fungi.

Certain outstanding differences between the average utilization of amino acids by individual organisms are worth mentioning. For the majority of the test-fungi histidine supports relatively little growth, but the secondary mycelium of *F. lividus* utilizes it almost as much as it utilizes proline and arginine. Asparagine, generally considered 'good' in growth supporting ability, appears intermediate in case of *P. cinnabarinus*. Despite the commonly observed nutritional superiority

of a complete series of amino acids, as in casein hydrolysate, the results show as much growth on average, with proline or with arginine or with asparagine singly in the basal medium as with casein hydrolysate in case of *P. cinnabarinus* and *P. zonalis*. While the reason for this is not clear, presumably certain environmental factors are more nearly optimal for assimilation of the single compound than for the mixture of amino acids.

Quantitative differences in nitrogen assimilation have been a general finding in studies of fungal nutrition. Presumably, both environmental factors and intrinsic differences in molecular structures may be involved in the differences in utilization of nitrogen compounds as has been observed in the present study.

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