

STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI.
XV. EFFECT OF DIFFERENT CARBON SOURCES ON
THE GROWTH OF *PYCNOPORUS SANGUINEUS*
(L. EX. FR.) MURR., *TRAMETES CINGULATA*
BERK. AND *DÆDALEA FLAVIDA* LE'V.

BY

PARBATI SAMAJPATI AND NIRMALENDU SAMAJPATI

*Mycology (Basic & Applied) Laboratory, Department of Botany,
Calcutta University, Calcutta-700019.*

The present study provides information about the role of different sources of carbon as a nutritional factor on the vegetative growth of three species of Basidiomycetes. Three fungi, one from each of the genus *Pycnoporus* (*P. sanguineus* (L. ex. Fr.) Murr.), *Trametes* (*T. cingulata* Berk.) and *Dædalea* (*D. flavida* Le'v.) were selected. To find out the role of carbon on the growth of the test-fungi, different sources of carbon including sugars and organic acids were used. Of these, sucrose and citric acid were found to be the best sources of carbon for growth.

INTRODUCTION

In the present investigation, the role of carbon sources on the vegetative growth of three species of wood-rotting Basidiomycetes has been recorded. The fungi under consideration belong to three allied genera, *Pycnoporus*, *Trametes* and *Dædalea* of the family Polyporaceæ. Of each genus, one species, common in West Bengal, viz. *Pycnoporus sanguineus* (L. ex. Fr.) Murr., *Trametes cingulata* Berk. and *Dædalea flavida* Le'v. were selected. This investigation is a contribution to the series of 'Physiology of Higher Fungi' published earlier.

A wide range of natural organic compounds are being utilized by fungi for the synthesis of structural and functional compounds and as sources of energy. Though some of them can utilize chemically complex carbon-containing compounds yet others are rather selective in their requirements.

Cambell (1932) has stated that some of the wood-rotting Basidiomycetes are able to decompose cellulose in the substratum. According to Blumer (1937), glucose is the best source of carbon for growth of *Ustilago violacea*. La Fuge (1937) has shown that *Polystictus versicolor* can utilize saponin (glucoside) due to the presence of oxidase enzymes. Schmitz and Kaufert (1938) have reported that

Lenzites trabea can cause greater amount of decay than *Lentinus lepideus* in sawdust soaked in sugar solutions than in sawdust moistened with distilled water. Hirayama (1938) has observed carbon requirements of three species of *Fomes* and three species of *Polyporus* and concluded that mainly hexoses and pentoses are the best sources of carbon. Herrick (1940) has demonstrated that glucose, fructose, mannose and galactose are good sources of carbon for growth of two isolates of *Stereum gausapatum*, of which one grows better on fructose.

Findlay (1941) has opined that *Lentinus lepideus*, *Coniophora cerebella*, *Merulius lacrymans* and *Poria vaillantii* cause less destruction of wood in the presence of added sugars. Johnson and Jones (1941) have stated that mannose is found to be the best source of carbon for *Coprinus* sp. It has been found that *Psalliota bisporus* grows best in the presence of xylose (Treschow, 1944). Cheo (1949) has observed that some of the isolates of *Ustilago striiformis* fail to grow on glucose-containing medium when freshly transferred from that containing sucrose. Lindeberg (1946) has shown that some wood-rotting fungi can utilize cellulose as source of carbon by decomposing the same. De Baun, Kudzin, and Schuberts (1950) have reported that *Lentinus lepideus* and *Trametes cinnabarina* are able to form oxalic acid if α -ketoglutaric acid is supplied in the medium. Rees and Levinson (1952) have demonstrated that some wood-rotting fungi can produce necessary enzymes to decompose natural cellulose for their nutrition. Tichy (1953) has investigated the effect of organic acids on growth of six species of Polyporaceæ and concluded that 2.5 to 4 per cent concentrations of acetic acid are unable to do any inhibitory effect on these fungi. Sucrose (5 per cent) has been found to be the best source of carbon for growth of four species of *Lenzites* (Mizumoto, 1956). Robbins and Harvey (1963) have found that ferrulic acid is a new co-factor for growth of *Polyporus schweintzii*. Samajpati and Banerjee (1971) have shown that sucrose and citric acid are the best sources of carbon for growth of *Fomes* (3 spp.) and *Ganoderma* (3 spp.). Mir *et al.* (1972) have shown that *Lenzites trabea* can produce oosponol and eburicoic acid in the medium.

MATERIAL AND METHODS

For the present investigation fungi were collected from Calcutta and its suburbs.

From freshly collected basidiocarps of all the fungi, monokaryophasic and dikaryophasic mycelial cultures were made following the method of Smith (1954). These were subcultured, maintained as stock cultures and used during the present investigation.

As stated in details by Samajpati and Banerjee (1967), glucose-casein hydrolysate medium (Leonian and Lilly, 1945) was selected for the purpose.

The standard basal medium has been first prepared without any carbon source. After preparation, the following sources of carbon have been separately added to each medium in several flasks as 20 gm. per litre. Malic acid, citric acid, succinic acid, oxalic acid, acetic acid, glucose, sucrose, fructose, galactose, maltose, xylose, raffinose and starch. The media have then been sterilized separately, inoculated separately by both the monokaryophasic and dikaryophasic mycelia of the test-fungi and incubated in the usual way. Several inoculated flasks with the medium, without any carbon source have been kept as controls. Other necessary experimental procedures have however, remained the same as described in Samajpati and Banerjee (1971).

RESULTS

The results obtained during the experimental period have been given in Tables 1-3. The data showing statistical analysis of variance have been shown in the Table 4.

Table 1. *Data (mean) showing the effect of different carbon sources on the vegetative growth (mg.) of the monokaryophasic and dikaryophasic mycelia of Pycnoporus sanguineus under different incubation periods.*

Source of Carbon	Incubation periods (days)					
	7		14		21	
	M*	D**	M*	D**	M*	D**
Glucose	70.00	93.00	91.31	116.25	123.00	146.16
Fructose	60.50	79.00	83.16	97.27	115.31	132.16
Galactose	50.00	68.00	69.00	90.16	100.67	125.37
Xylose	48.30	66.10	63.35	83.37	89.16	115.16
Maltose	57.00	74.00	78.35	96.16	99.19	121.40
Sucrose	63.90	85.95	82.36	104.35	103.10	129.16
Raffinose	53.00	70.00	74.30	89.16	90.24	118.16
Starch	44.00	61.15	62.10	80.00	81.26	100.37
Citric acid	67.00	90.00	88.16	111.26	109.00	132.16
Oxalic acid	51.00	64.00	70.20	83.05	89.16	101.31
Acetic acid	57.16	71.25	75.00	90.00	93.00	108.25
Malic acid	61.17	76.18	80.00	98.16	100.25	117.36
Succinic acid	64.00	88.31	83.25	104.35	106.36	121.25
Control	40.00	60.27	58.16	76.37	78.17	96.46

* Monokaryophasic mycelia.

** Dikaryophasic mycelia.

Table 2. Data (mean) showing the effect of different carbon sources on the vegetative growth (mg.) of the monokaryophasic and dikaryophasic mycelia of *Trametes cingulata* under different incubation periods.

Source of Carbon	Incubation periods (days)					
	7		14		21	
	M*	D**	M*	D**	M*	D**
Glucose	67.25	90.75	87.16	110.65	119.18	139.00
Fructose	57.25	74.00	80.25	94.27	111.30	126.75
Galactose	48.76	63.75	64.00	86.17	95.65	116.00
Xylose	47.00	60.25	60.00	80.00	82.00	104.65
Maltose	51.36	70.00	72.00	90.16	91.20	114.26
Sucrose	62.26	84.25	80.25	100.00	98.36	119.75
Raffinose	50.00	67.00	70.17	85.25	85.00	110.65
Starch	41.75	60.00	59.16	76.25	76.75	94.75
Citric acid	63.00	86.25	83.75	105.16	100.00	128.26
Oxalic acid	50.50	60.17	66.36	78.75	82.75	96.25
Acetic acid	56.17	66.36	71.00	87.25	88.65	98.10
Malic acid	57.00	71.27	74.25	94.00	93.75	109.26
Succinic acid	60.16	84.75	79.36	98.75	98.25	115.00
Control	33.00	53.17	51.25	70.37	71.27	91.75

* Monokaryophasic mycelia.

** Dikaryophasic mycelia.

Table 3. Data (mean) showing the effect of different carbon sources on the vegetative growth (mg.) of the monokaryophasic and dikaryophasic mycelia of *Dædalea flavida* under different incubation periods.

Source of Carbon	Incubation periods (days)					
	7		14		21	
	M*	D**	M*	D**	M*	D**
Glucose	76.15	100.00	97.36	125.00	130.17	156.00
Fructose	65.75	85.00	90.00	104.00	120.25	140.00
Galactose	56.15	75.00	75.00	96.00	107.00	132.00
Xylose	54.26	72.16	69.75	90.26	96.26	122.00
Maltose	63.00	80.25	84.16	103.00	105.25	128.00
Sucrose	70.00	92.26	86.00	110.00	111.00	136.00
Raffinose	58.25	76.76	80.00	96.00	96.25	125.00
Starch	50.00	67.00	69.00	87.00	90.00	110.25
Citric acid	75.00	97.00	93.00	118.00	115.00	140.00
Oxalic acid	57.00	70.75	75.00	90.00	95.00	109.00
Acetic acid	64.25	78.00	80.00	97.00	99.00	115.00
Malic acid	67.00	84.36	85.00	105.00	106.00	124.50
Succinic acid	70.00	95.00	89.00	110.75	111.00	128.75
Control	46.00	67.25	62.25	82.16	80.17	100.26

* Monokaryophasic mycelia.

** Dikaryophasic mycelia.

Table 4. *Analyses of variance table showing the effect of carbon on the growth of the three test-fungi at different incubation periods.*

Source of variation	d.f.	m.s.s.	F-value
Fungus	5	17255	140.28*
Carbon	13	6620.16	53.82*
Incubation period	2	112784.5	916.94*
Fungus × Carbon	65	29.98	
Fungus × Incubation period	10	49.5	
Carbon × Incubation period	26	216.80	1.76
Error	634	123	
Total	755		

* Significant at 1 percent level of P.

From the foregoing Tables (1-4) it is evident that sucrose is the best source of carbon for all the species under consideration. Glucose, maltose and fructose are also found to be suitable for growth but next in importance to sucrose while galactose, xylose and starch have but poor effects. Raffinose has also been found to have some effect on vegetative growth of all the three test-fungi.

Among the organic acids, citric acid comes first as the best source of carbon. Next come malic acid and succinic acid in succession while due to the presence of oxalic acid and acetic acid the effect on vegetative growth appear to be minimum in all cases.

DISCUSSION

After evaluating the role of different sources of carbon on the growth of all the test-fungi, it has been noted that glucose and citric acid are the best sources among the sugars and the organic acids respectively. Next to glucose, sucrose has appeared to be the best source of carbon followed by fructose, maltose and raffinose in order of sequence. Glucose has been directly taken up by the fungi for further metabolism. Sucrose has been possibly broken down into fructose and glucose and maltose into two molecules of glucose. The growth data have suggested that the required enzymes, namely, β -fructofuronosidase and α -glucosidase, must have been produced by all the test-fungi. Raffinose has been probably utilized by the enzyme raffinase. The poor effect of galactose has appeared to be possibly due to failure to convert galactose to glucose-6-phosphate for some unknown reasons. Xylose and starch must have not been properly broken down due to lack of the specific enzymes. Among the organic acids, all the test-fungi have been able to utilize the respective acid of Tricarboxylic acid-cycle as source of carbon. This fact has indicated that all the

test-fungi possibly use the TCA-cycle for metabolism of organic acids (Barnett and Kornberg, 1960). The available growth data on citric acid, succinic acid and malic acid have strongly suggested this idea.

REFERENCES

- Barnett, J. A., and Kornberg, H. L. (1960). The utilization by yeasts of acids of tricarboxylic acid cycle. *J. Gen. Microbiology*, 23: 65-82.
- Blumer, S. (1937). Untersuchungen über die Biologie von *Ustilago violacea* (Pera) Fuck. I. Mitteilung: Ernährungs- und Kulturbedingungen. Wirkungen des Saponins. *Arch. Mikrobiol.*, 4: 458-478.
- Campbell, W. G. (1932). The chemistry of white rots of wood. III. The effect on wood substances of *Ganoderma applanatum* (Pers.) Pat., *Fomes fomentarius* (L.) Fr., *Polyporus adustus* (Willd) Fr., *Pleurotus ostreatus* (Jacq.) Fr., *Armillaria mellea* (Vahl.) Fr., *Trametes pini* (Brot.) Fr., *Polystictus abietinus* (Dicks.) Fr., *Biochem. J.*, 26: 1829-1838.
- Cheo, P. C. (1949). Thesis, West Virginia University, cited by Lilly, V. G., and Barnett, H. L. (1951), in *Physiology of Fungi*, McGraw-Hill Book Co. USA.
- De Baun, R. M., Kudzin, S. F., and Schubert, W. J. (1950). On the mechanism of enzyme action. XLI. A role of α -ketoglutaric on the carbohydrate metabolism of wood-destroying molds. *Arch. Biochem.*, 26: 375-381.
- Findlay, W. P. K. (1941). Effect of addition of sugars on rate of decay of wood. *Ann. Appl. Biol.*, 28: 19-22.
- Herrick, J. A. (1940). The carbon and nitrogen metabolism of *Stereum gausapatum* Fr., *Ohio J. Sci.*, 40: 123-129.
- Hirayama, S. (1938). Studies on the metabolism of some wood-rotting fungi. *Ann. phytopath. Soc., Japan.* 8: 19-21.
- Johnson, G. T. and Jones, A. C. (1941). *Mycologia*, 33: 424-433, cited by Cochrane, V. W. (1958). *Physiology of Fungi*, John-Wiley & Sons. Inc. N. Y.
- La Fuze, H. H. (1937). Nutritional characteristics of certain wood-destroying fungi, *Polyporus betulinus* Fr., *Fomes pinicola* Fr., Cooke and *Polystictus versicolor* Fr., *Plant Physiol.*, 12: 625-646.
- Leonian, L. H. and Lilly, V. G. (1945). The comparative value of different test organisms in the microbiological assays of B vitamins. *West Va Agric. Expt. Sta. Bull.* No. 319.
- Lindeberg, G. (1946). On the decomposition of lignin and cellulose in litter caused by soil inhabiting Hymenomycetes. *Ark. for. Botanik.*, 33: 1-16.
- Mir, I., Ahmad, S., and Hamid, A. (1972). Chemical investigation on *Lenzites trabea*. *Pakistan Journal of Scientific & Industrial Research*, 14: 479-480.
- Mizumoto, S. (1956). Studies on *Lenzites abietina* Fr., and some of its allied species. VI. Effect of sodium pentachlorophenate upon *L. abietina*, *L. subferruginea* Berk., *L. trabea* (Pers) Fr., and *L. saepiaria* (Wulf) Fr., *J. Jap. For. Soc.*, 38: 111-113.
- Reese, E. T. and Levinson, H. S. (1952). A comparative study of breakdown of cellulose by micro-organisms. *Physiol. Plantarum*, 5: 345-366.
- Robbins, W. J. and Harvey, A. (1963). Growth factors for *Polyporus schweinitzii*. The identification of Ferulic acid as a new co-factor. *Mycologia*, 55: 742-757.
- Samajpati, N., and Banerjee, S. N. (1967). Studies on the physiology of higher-fungi. I. Fatty acids as carbon sources of three species of *Fomes* and *Ganoderma*. *Indian J. mycol., Res.*, 5: 40-44.
- , and ———. (1971). Studies on the physiology of higher-fungi. IV. Effect of different carbon sources, on vegetative growth of some species of *Fomes* and *Ganoderma*. *Proc. Indian Nat. Sci. Acad.*, 37: 68-73.
- Schmitz, H., and Kaufert, F. (1938). Studies in wood decay. VIII. The effect of the addition of dextrose and dextrose and asparagine on the rate of decay of Norway Pine sapwood by *Lenzites trabea* and *Lentinus lepideus*. *Amer. J. Bot.*, 25: 443-448.
- Smith, G. (1954). *An Introduction to Industrial Mycology* (4th Ed.) Edward Arnold, London.
- Tichy, V. (1955). Podíl Kyseliny unnirove na fungistatických vlastnostech lišejníku *Usnea hirta* Hoffm. Studie o provoze lišejníkových antibiotik. *Publ. Fac. Sci. Univ. Masaryk.*, 364.
- Treschow, C. (1944). Nutrition of the cultivated mushroom, *Dank Bot. Arch.* 11: 1-180.

(Accepted for publication 20th March 1975)