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The metabolism of Pleurotus ostreatus (JACQ. ex FR.) KUMMER, Pleurotus florida BLOCK and Pleurotus cornucopiae PAUL. ex FR. from taxonomical and other viewpoints

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ABSTRACT: The author examined the carbon metabolism in Pleurotus ostreatus, Pleurotus florida and Pleurotus cornucopiae in some respects and also their utilization of nutrient taken from waste materials of vegetable origin. According to the results, all the three species have somewhat deviating characteristics. From the viewpoint of nutrient utilization, Pleurotus florida is the quickest, the comes Pleurotus ostreatus and the slowest is Pleurotus cornucopiae. The author examined to what extent the nutrient physiological data support the debated question of Pleurotus florida being as independent species.

The cultivation of various Pleurotus species under laboratory conditions was examined. In addition the utilization of various carbon sources of nutrients in three Pleurotus species was investigated as a chemotaxonomical characteristic this value was to be used in accurate evaluations of the species. Therefore, the examination of the carbon material mechanism of Pleurotus species became necessary in certain respects (for example, sugar utilization).

Another objective of our laboratory examinations was to evaluate the nutrient values of materials like factory wastes used on a large scale as well as wastes of vegetable origin obtained from other, agricultural and light-industrial plants with a view to finding possibilities for their use as nutrient materials of Pleurotus species, on altogether 6 kinds of culture media.

Cultivation - The cultivation of Pleurotus ostreatus on tree trunks and logs was first described at the beginning of the 20th century by Anonymus (1919, 1924), Brasch (1921), Busse (1920), Falck (1917, 1919), Luthard (1969), Passecher (1959), Véssey and Tóth (1969). (ZADRAZIL 1974). The experimen-

tation in growing Pleurotus ostreatus in artificial culture medium was begun between 1945 and 1950. (LABORDE-DELMAS 1974).

The industrial bases for producing the substrate and the fruit bodies were developed by Heltay et al. (1971), Junkova (1971), Kalberer and Vogel (1974), Stanek and Rysava (1971), Zadrazil and Schneidereit (1972), Zadrazil (1973). (ZADRAZIL 1974). Eger imported Pleurotus florida, and it was first grown in 1963, in Max-Planch Institute (RUTTEN 1975).

Material and method - Investigations were carried out on 6 kinds of substrates with 3 Pleurotus species. The substrates were collected by selection from waste-materials obtained from agricultural and light-industrial factories.

The substrates were as follows:

I. Of factory origin (the composition of the substrate used on a large scale at present is: wheat-straw: corn-cob 3:7)
II. Corn-cob (Zea mays)
III. Corn stalk (Zea mays)
IV. Poplar saw-dust (Populus sp.)
V. Sorghum stalk (Sorgum sp.)
VI. Barley-straw (Hordeum sp.)

Stages of preparing the substrate:

Drying, crushing, soaking, pouring into petri-dishes, sterilization in autoclave, at 1 atm pressure for about 30 minutes. Grafting: the sterilized substrates were grafted in grafting chambers, under sterile conditions.

The maintenance of the cultures: During the period of interweaving they were kept at constant temperature, in a thermostat at 22 C^{o}. After complete interweaving they were placed into such chambers where the quantity of light, air, and temperature necessary for the formation of the fruit bodies were ensured. The materials grafted with Pleurotus ostreatus were placed for several days into a chamber with temperature at 5-6 C^{o} for them to undergo the necessary cold effect.

The examined parameters - We examined to what extent some strains and the same strain utilize certain substrates. We measured the rate of interweaving and determined the sugar, lignin and cellulose content. The rate of interweaving was determined by measuring the diameter of the thallus at 3-4 day intervals, up to the time of full interweaving. To carry out investigations into the internal contents, samples were taken from the initial materials, then from the cultures on three occasions. The first sample was taken after the interweaving started, the second sample on the appearance of the fruit bodies, the third sample after cutting of crops.

Method of evaluation - 1. Determination of total sugar content (Anthron method). The sugar component or components with the anthron in sulphuric acid medium produced a greenish-blue colour. The intensity of the colour obtained depends on temperature, period of warming, and on the concentration of sulphuric acid and anthron. 2. The determination of the filament content was carried out in two phases. First we determined the lignin content, then the cellulose content, on the basis of Edwards' method.

Result of the examinations - By measuring the rate of growth it could be stated (Table 1) that of the three species Pleurotus florida showed the highest

growth rate, while Pleurotus cornucopiae showed the lowest rate. In all three species, the interweaving in saw-dust took the longest time. The various fungi took the shortest time, almost the same, to interweave the substrate of the factory mixture, and that of corn stalk and straw. The structure of mycelium was different on the individual substrates in the case of all three culture stocks. It was Pleurotus florida that took the least time to produce fruit bodies in all the cases. Until the time of closing the examinations, on the specimens grafted with Pleurotus cornucopiae no fruit bodies appeared. Also according to the data of the literature, the time to produce fruit bodies is longer than that of the other two species, but possibly other technical factors also contributed to its not having produced fruit bodies.

Table 1. Measurement data of growth rate (cm) in the individual substrates as a function of time. 1 = Pleurotus ostreatus, 2 = Pleurotus florida, 3 = Pleurotus cornucopiae, += interweaving has taken place

sub-strate	Fungus	Days							
		4.	7.	10.	14.	18.	21.	24.	28.
I	1	1.6	3.8	8.5	+				
	2	1.4	4.2	9.1	+				
	3	1.4	2.7	4.3	7.7	+			
II	1	1.7	2.9	4.7	8.6	+			
	2	1.6	3.6	5.4	9.4	+			
	3	1.3	2.5	4.2	6.7	11.7	+		
III	1	2.7	4.8	7.4	+				
	2	3.1	5.1	7.8	+				
	3	1.9	3.4	4.6	7.6	+			
IV	1	1.4	2.3	3.6	4.7	6.3	8.9	+	
	2	1.4	2.8	3.9	5.5	9.2	+		
	3	1.3	2.1	2.9	3.7	5.1	7.2	9.1	+
V	1	1.7	3.2	6.6	9 3	+			
	2	1.8	4.6	7.7	9.7	+			
	3	1.4	2.3	5.2	6.7	11.5	+		
VI	1	1.5	3.9	9.8	+				
	2	1.6	5.3	10.4	+				
	3	1.3	2.4	6.7	8.0	+			

I = factory mixture, II = corn cob, III = corn stalk, IV = poplar saw-dust, V = sorghum stalk, VI = barley-straw

According to the analysis of the internal contents (Table 2), of the 6 kinds of substrates corn stalk and Sorghum stalk have very high initial free sugar content, while lignin and cellulose are contained in saw-dust in the greatest quantity.

Table 2. Internal contents of the initial substrates mg/lg dry matter (for I-VI see Table 1)

Substrate	Sugar	Lignin	Cellulose
I	99.3	95.6	463.4
II	78.6	122.0	480.9
III	193.0	123.9	442.6
IV	75.3	238.4	495.4
V	212.0	130.4	386.5
VI	155.6	126.2	377.2

In Pleurotus ostreatus a similarity expressed on the basis of all the parameters examined was shown between the factory mixture and corn stalk (Tables 3 and 5). This species was of the highest mycelium yield among the 3 species. The times of interweaving did not differ too much in the individual substrates (Table 1), but mycelium production was different during identical times. It was only the thallus diameter that could be measured, the quantity of mycelium could not be measured because it cannot be isolated from the substrate, but in the same time of mycelium production, the utilization of corn stalk proved to be the best. The substrates of the factory mixture and of sorghum stalk were also quite markedly interwoven. The substrate of saw-dust was the least interwoven. In Pleurotus florida, smaller differences occurred in the individual culture media. This species was the earliest to produce fruit bodies. Its breaking down of the cellulose was much more considerable in the phase of producing the fruit bodies than was that of Pleurotus ostreatus. Parallel with the high rate of cellulose break-down, the quantity of sugar also increased in the medium. The quantity of sugar, apart from two exceptions, decreased in the beginning, then, in the course of the second sampling, increased at first to a greater extent and then to a lesser one. The initial decrease can probably be explained by the fact that fungi first mainly take up the available free sugar. Cellulose is broken down by them into D-glycose. In the course of the second sampling, the activity of the enzymes breaking down cellulose abruptly increases, which is probably induced by the decrease in the sugar content. In specimens grafted with Pleurotus cornucopiae analyses were carried out only after the interweaving had taken place. At this phase the lignin decomposed the most in the three species. Comparing the individual substrates, we can say that the degree of their utilization depends on the quantity and ratio of their main components (lignin, cellulose, sugar) as well as on their structure (grain size, compactedness). Saw-dust, although it has the highest lignin and cellulose content (Table 2), has not proved to be so satisfactory a substrate due to its compact structure, as for example the corn stalk of looser fibres.

Table 3. The internal contents of samples taken from substrates of factory-mixtures on three occasions during the cultivation

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	88.7	76.7	62.0
	Cellulose	428.7	453.0	438.3
	Sugar	45.0	66.3	58.3
2	Lignin	75.6	72.3	
	Cellulose	381.0	377.6	
	Sugar	182.6	218.6	
3	Lignin	71.5	68.0	
	Cellulose	339.2	339.0	
	Sugar	284.3	299.3	

Table 4. The internal contents of samples taken from corn cob on three occasions during the cultivation

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	87.0	64.1	57.6
	Cellulose	420.7	428.1	439.3
	Sugar	46.3	76.0	70.0
2	Lignin	84.4	52.2	
	Cellulose	406.2	374.8	
	Sugar	144.3	202.3	
3	Lignin	71.7	43.1	
	Cellulose	402.1	364.2	
	Sugar	156.6	303.0	

Table 5. The internal content values of samples taken from corn stalk on three occasions during the cultivation

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	121.2	99.8	92.0
	Cellulose	406.6	388.6	397.7
	Sugar	134.0	396.0	267.6
2	Lignin	113.5	94.8	
	Cellulose	315.8	275.6	
	Sugar	279.0	444.3	
3	Lignin	105.1	91.0	
	Cellulose	290.5	248.2	
	Sugar	371.0	480.1	

Table 6. The internal content values of samples taken from sawdust on three occasions during the cultivation

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	218.4	205.6	201.2
	Cellulose	464.2	485.5	458.0
	Sugar	44.0	43.3	53.0
2	Lignin	216.4	203.2	
	Cellulose	444.1	455.1	
	Sugar	71.0	49.6	
3	Lignin	204.5	198.9	
	Cellulose	417.3	437.2	
	Sugar	79.0	80.0	

Table 7. The internal content values of samples taken from sorghum stalk on three occasions during the cultivation

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	127.8	102.2	81.9
	Cellulose	334.5	367.4	323.5
	Sugar	195.6	190.5	257.6
2	Lignin	120.4	94.0	
	Cellulose	305.9	304.3	
	Sugar	350.0	386.6	
3	Lignin	97.5	70.3	
	Cellulose	277.7	183.0	
	Sugar	461.2	494.3	

Table 8. The internal content values of samples taken from barley straw on three occasions during the experiment

Sampling	Material/mg	Fungi		
		Pl. ostr.	Pl. flor.	Pl. corn.
1	Lignin	113.0	100.8	86.9
	Cellulose	366.5	355.3	361.0
	Sugar	145.0	133.0	94.0
2	Lignin	107.7	98.2	
	Cellulose	307.9	279.4	
	Sugar	286.5	310.6	
3	Lignin	104.5	95.0	
	Cellulose	281.7	233.4	
	Sugar	329.2	377.0	

SUMMARY

1. The mycelium growth and its turning into fruit in the fungus species examined influenced by the substrates of cultivation.

2. The rate of interweaving was fastest in the corn stalk then the factory mixture and straw (Table 1). In these, the initial free sugar content is high (corn stalk, straw), or the cellulose content is high (factory mixture).

3. The utilization of saw-dust, although this is the natural substrate of fungi, is not rapid, according to our examinations; the mycelium growth is slow and its production is low, in spite of the high cellulose and lignin content (Table 6).

4. It can be inferred that all the three Pleurotus species utilize the sugar content of the substrate at the initial phase of the interweaving, influenced to a certain extent by the initial composition of the substrate.

5. The decomposition of the lignin in Pleurotus florida is always the most intensive in the phase of interweaving, which at the phases slowly decreases. The lignin utilization in Pleurotus ostreatus in most substrates is similar to that of Pleurotus florida, but in sorghum stalk the lignin utilization increased also during the formation of fruit bodies.

6. The decomposition of cellulose in Pleurotus florida is highest just before the formation of fruit bodies, irrespective of the substrate, while in Pleurotus ostreatus the highest decomposition of cellulose occurred during the phase of interweaving, in sorghum stalk and corn cob.

7. The fungus species examined are rather cellulolytic than lignin utilizers, this is especially true for the physiological phase of interweaving and for that preceding the formation of fruit bodies.

8. Beside the morphological and ecological differences between Pleurotus ostreatus and Pleurotus florida, the latter resembles in structure that of Pleurotus ostreatus, but is somewhat smaller and finer. The cap colour is pale yellow, whitish, as against of the greyish-brown, slate-grey colour of Pleurotus ostreatus' cap (ZADRAZIL 1974). The formation of Pleurotus ostreatus' fruit bodies requires cold-treatment of about 10 days, at 5-6 ^{o} C (ZADRAZIL-SCHNEIDEREIT 1972); the species deviations can be detected also in carbon metabolism. The breaking-down of substrates is basically similar, but in Pleurotus ostreatus, the degree of cellulose and lignin decomposition is more influenced by the ratio of compositions of the initial substrates, than in Pleurotus florida.

9. Species differences occur also in the substrate utilization, which is shown by the Pleurotus florida's faster interweaving and producing of fruits.

10. Pleurotus cornucopiae in our examinations differed from the other two by its greatest initial lignin consumption. The degree of its cellulose utilization is at the same time similar to that of Pleurotus ostreatus. Its initial sugar consumption is smaller compared with that of the others, while its lignin utilization at the same time is higher.

11. Considering the rate of interweaving and the morphological picture of mycelium in Pleurotus cornucopiae, the nutrient utilization is not so intensive as that other two species.

12. The results of these examinations, i.e. the ratio of the utilization of main internal contents, the rate of interweaving, the production of mycelium,

the time of appearance of the fruit bodies in Pleurotus florida provide certain information to support its being an independent species.

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