THE DECOMPOSITION OF CELLULOSE BY SOIL FUNGI*

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Probably the most abundant energy source available to soil fungi is cellulose. All fungi do not have the same capability of breaking down this material because the enzyme cellulase is not produced by all fungi. Schubert and Nord (1950) have shown that certain fungi can preferentially decompose cellulose in wood, leaving the lignin. Mandels and Reese (1960) found that the synthesis of the enzyme cellulase could be induced in some fungi when grown upon cellobiose.

This study was made of selected soil fungi, particularly species of *Aspergillus* and *Penicillium*, to find which species decompose cellulose and what factors affect its decomposition.

METHODS AND MATERIALS

For laboratory investigations, the organic matter was destroyed in a Xenia silt loam by hydrogen peroxide oxidation. The following mineral salts were placed in each flask: 200 mg KH_2PO_4, 500 mg $Ca(OH)_2$, 100 mg $MgSO_4$. Lignin, cellulose, and a lignin-casein complex were also added. Each flask contained a total of 80 gm of material.

The medium used to determine cellulose digestion by specific fungi consisted of: 5 g Whatman cellulose powder, 1.1 g KH_2PO_4, 0.1 g $MgSO_4$, and 0.1 g NH_4Cl per liter.

Nitrogen determinations were made by micro-Kjeldahl.

In the first experiments, flasks containing 80 g of sterile soil-organic matter additions were innoculated with 0.1-ml suspensions of the following organisms: *Bacillus cereus*, *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Hormodendrum* sp., *Fusarium* sp., and *Rhizopus nigricans*. The flasks were kept at 20 percent moisture content by weighing and by additions of sterile water and incubated at room temperature for 20 weeks. After this 20-week period 10 g-samples of soil were taken for plate counts. Counts of fungi were made in triplicate on rose bengal agar (1944). To determine which of the fungi initially innoculated had the ability to decompose cellulose, species isolated from the plates in the first experiment and other selected organisms were innoculated into a liquid medium containing cellulose as the only source of carbon. The medium was adjusted to a pH of 5.6 and after a period of 14 days the mycelium was harvested by filtering onto small disks of filter paper. The nitrogen content was then determined.

RESULTS AND DISCUSSION

A 2 year study has been made of selected soil fungi, especially of the genera *Penicillium* and *Aspergillus*, to determine which of these organisms could decompose cellulose. One test organism was *Penicillium ochro chloron*, a known cellulose decomposer, which was obtained from the Northern Research Laboratory, Peoria, Illinois. Three other organisms, *Lenzites saepiaria*, *Poria vaillantii* and *Lentinus lepideus*, were obtained from the A. T. C. C. These latter organisms were the ones used by Schubert and Nord (1950) in an attempt to concentrate the amount of lignin present in wood samples by allowing these organisms to decompose the cellulose.

In an initial experiment, counts of fungi made after a 20-week incubation period showed rather large differences depending upon the organic substrate added

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TABLE 1

The number of fungi isolated from a basic soil medium with various soil amendments

Treatment	Organisms	Number/g "x10²"	Final pH
Check plus 0.17 g NH_4NO_3	*Penicillium*	50	6.6
	Fusarium	70	
	Undetermined	125	
	Total	245	
Lignin[1] Plus 0.17 g NH_4NO_3	*Penicillium*	215	4.8
	Fusarium	10	
	Undetermined	190	
	Total	415	
Lignin plus 0.33 g NH_4NO_3	*Penicillium*	415	4.6
	Fusarium	15	
	Undetermined	470	
	Total	900	
Cellulose plus 0.17 g NH_4NO_3	*Penicillium*	110	4.9
	Fusarium	335	
	Undetermined	910	
	Total	1355	
Casein-Lignin[2] complex (0.19 g N in complex)	*Penicillium*	15	6.6
	Fusarium	550	
	Undetermined	2135	
	Total	2700	

[1]All organic additions at the rate of 4 g. Total weight of material in each flask was 80 g.

[2]This complex was prepared by adding four parts of lignin with one part of casein.

TABLE 2

Amount of growth on cellulose medium as determined by nitrogen content of mycelium

Organism	pH	[1]Mg N in mycelium
Fusarium sp.	4.1	0.25
	5.2	0.61
	6.3	1.29
[2]*Aspergillus* sp.	4.1	0.00
	5.2	0.00
	6.3	0.00
[2]*Penicillium* sp.	4.1	0.00
	5.2	0.00
	6.3	0.00
Trichoderma viride	5.2	1.31
Trichoderma viride	6.3	0.84

[1]28 days growth.

[2]No nitrogen found at other pH values tested.

to the flasks (table 1). A rather large increase in numbers of *Fusarium* sp. in proportion to *Penicillium* sp. was obvious when cellulose was added.

In table 2 it can be noted that *Fusarium* sp. and *Trichoderma viride* utilize cellulose as a source of carbon and that the *Penicillium* sp. does not. This probably explains the higher counts of *Fusarium* in table 1 when cellulose was added. It should also be noted that the amount of growth as indicated by the nitrogen content of the mycelium increased with increasing pH in the case of *Fusarium* sp. but decreased with pH in the case of *Trichoderma viride*.

TABLE 3

Amount of cellulose decomposition by species of soil* Penicillia *and* Aspergilli

Organism	*Mg N	Organism	*Mg N
[1]*P. ochro chloron*	2.27	*P.* 32	1.13
P. commune	0	*P.* 33	0
P. chrysogenum	0	*P.* 34	0
P. cyclopium	0		
P. rugulosum	0	*A. oryzae*	0
P. adametzi	0.40	[1]*A. terreus*	1.28
P. citrinum	0.15	*A.* 1	0
[2]*P.* 1	0	*A.* 2	0
P. 2	0	*A.* 4	0.95
P. 3	0	*A.* 5	0
P. 4	0	*A.* 6	0
P. 5	0.82	*A.* 7	1.54
P. 6	0	*A.* 8	0
P. 7	0	*A.* 10	0
P. 8	0	*A.* 11	0
P. 9	0	*A.* 13	0
P. 10	0	*A.* 15	0
P. 11	1.80	*A.* 16	1.20
P. 12	0.93	*A.* 17	1.40
P. 13	0.76	*A. 18*	0
P. 14	1.83	*A. 19*	0
P. 15	1.47		
P. 16	0.77	*Lenzites saepiaria*	0
P. 17	0.68	*Poria vaillantii*	0
P. 18	0.93	*Lentinus lepideus*	0
P. 19	0		
P. 21	0.25	*Trichoderma viride*	1.83
P. 22	1.86		
P. 23	0	*Fusarium* 1	1.54
P. 24	0	*Fusarium roseum*	0.25
P. 25	0.95	*Fusarium* 2	0.60
P. 26	0	*Fusarium oxysporum* strain A	1.90
P. 27	1.03	*Fusarium oxysporum* strain B	1.46
P. 28	0	*Fusarium oxysporum* strain E	2.45
P. 29	0.25		
P. 30	1.81	*Streptomyces anulatus*	0
P. 31	0.49	*Streptomyces cellulosae*	0

*Decomposition was determined by nitrogen found in mycelium.
[1]Species obtained from the Northern Research Laboratories, Peoria, Illinois.
[2]Laboratory number of soil isolates.

Species capable of deomposing cellulose vary physiologically even within a given genus. This can be seen in the large differences in decomposition of this material among the species of *Penicillium* and *Aspergillus* (table 3). Of the 41 species of *Penicillium* tested for cellulose decomposition, only about one-half were able to utilize cellulose as a sole source of carbon. Only 4 of the organisms tested approached the rapid cellulose decomposition of cellulose as shown by *P. ochro chloron*, and none decomposed cellulose at a more rapid rate. Identification of

the 4 most rapid cellulose digestors showed them to be of the series *P. janthinellium*[1] as suggested by Raper and Thom (1949). Only 4 of the species of *Aspergillus* that were isolated from the soil decomposed cellulose. The number of species of *Aspergillus* isolated from the soil, however, was not nearly as great as those of *Penicillium*. The decomposition of cellulose in this medium by 3 known cellulose decomposers, namely *P. ochro chloron*, *A. terreus*, and *T. viride*, show that the medium is a reliable one to use for such studies. The three organisms used by Schubert and Nord (1950), *Lenzites* sp., *Poria* sp., and *Lentinus* sp. showed no utilization of cellulose on this medium, but have been reported to be cellulose digestors. These fungi did not decompose cellulose even when additional amounts of thiamine was added to the basic medium.

TABLE 4

The effect of various clays in the decomposition of cellulose by P. ochro chloron

Material		Mg N
Control		1.41
Kalonite	0.1 g*	1.10
"	0.5 g	0.88
"	1.0 g	0.84
Illite	0.1 g	1.07
"	0.5 g	1.05
"	1.0 g	0.99
Bentonite	0.1 g	0.86
"	0.1 g	0.86
"	0.5 g	0.55
"	1.0 g	0.54
Lignin	0.05 g	1.40
"	0.10 g	1.42

*Amount of material added to the basic medium.

This basic medium of inorganic salts and cellulose was successful with other known cellulose decomposers that were tried, including several species of the *Fusarium*. The use of this medium for distinguishing between cellulose digestors could be useful as a biochemical test for identifying certain species of *Penicillium*. Trials with the same organism grown upon this medium for identical lengths of time gave identical amounts of nitrogen incorporated in the mycelium. Cochrane (1958), in discussing methods for measuring cellulose decomposition, points out that perhaps the most reliable measurement for cellulose decomposition is the determination of total nitrogen in the insoluble material. In making plate counts as shown in table 1, it would be better to consider the types of organisms involved rather than just the total fungus count.

To test the effects of materials, like clay, that might influence the activity of the enzyme cellulase, different clays were added to the basic cellulose medium. If cellulase is a true induced enzyme, then compounds that could adsorb the enzyme or interfere with its function in other ways should reduce the utilization of cellulose by *P. ochro chloron*. Table 4 shows that clays do reduce the digestion

[1]Organisms *P.* 14, 22, 30, 11 have been identified by Agricultural Research Service, Peoria, Ill., as *P. janthinellium*, also *P.* 15 as *P. piscarium* and *P.* 17 as *P. frequentans*. These *Penicillium* can be easily separated biochemically by carefully using this basic cellulose medium and determining the nitrogen content of mycelium.

of cellulose by this organism, but the reduction is not proportional to the amount of clay added. Different clays had a different effect, but the initial reduction of cellulose utilization was greater for the first increment than when larger amounts of clay were added. Perhaps even small amounts of clay have sufficient surface area and exchange sites to adsorb all the enzyme synthesized or inhibit its activity. Lignin added to the medium had no effect on cellulose utilization by *P. ochro chloron*.

When cellulose was replaced with cellobiose as a source of carbon in the basic medium, species of *Penicillium* that did not utilize cellulose broke the β-glycosidic linkage of cellobiose (table 5). Factors other than the glycosidic linkage must be affecting the breakdown of cellulose. *P. ochro chloron* still was able to utilize this substrate much better than the other *Penicillium* tested. Mandel and Reese (1960) found that all materials inducing the synthesis contained the β-glycosidic linkage, but non-inducing compounds may also contain this linkage. When cellobiose octa acetate was substituted for cellulose as the substrate, only *P. ochro chloron* utilized this material. The amount of nitrogen incorporated into the mycelium of this organism was so small that it may have used parts of the molecule that were not completely acetylated. The presence of such acetyl groups on the sugar molecule makes it difficult for even rapid cellulose decomposers to utilize this substrate.

TABLE 5

Utilization of cellobiose and cellobiose octaacetate by selected species of Penicillium

Organism	Cellobiose Mg N	Cellobiose octaacetate Mg N
P. commune	3.18	0.0
P. ochro chloron	3.93	0.3
P. chrysogenum	1.66	0.0
P. 1*	3.12	0.0

*Laboratory number of *Penicillium* isolate from soil.

Two additional test organisms, *Streptomyces anulatus* and *Streptomyces cellulosae*, gave no decomposition of cellulose in the basic salts and cellulose medium. These organisms are listed as cellulose decomposers in Bergey's Manual (1957). The inability of 2 species of *Streptomyces* to utilize cellulose under these conditions may point to the importance of fungi as primary attackers of cellulose. Although all fungi cannot utilize cellulose, once this complex molecule is broken down to simpler components, it becomes available to a wider spectrum of organisms. The initial utilization of cellulose is possible by many species of *Aspergillus* and *Penicillium* occurring in the soil, so that its breakdown should be fairly rapid. The fungi have to be considered as an important group of organisms in the initial steps of cellulose decomposition.

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