APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1982, p. 23–27
0099-2240/82/070023-05$02.00/0
Vol. 44, No. 1

Degradation of Coal by the Fungi *Polyporus versicolor* and *Poria monticola*

MARTIN S. COHEN[1]* AND PETER D. GABRIELE[2]

Department of Biology and Health Science, University of Hartford, West Hartford, Connecticut 06117,[1] and CIBA-GEIGY Corporation, Ardsley, New York 10502[2]

Received 17 November 1981/Accepted 29 March 1982

We report that two species of basidiomycete fungi (*Polyporus versicolor* and *Poria monticola*) grow in minimal liquid or solid medium when supplemented with crushed lignite coal. The fungi also grow directly on crushed lignite coal. The growth of both fungi was observed qualitatively as the production and extension of hyphae. No fungal growth occurred in minimal agar medium without coal. The fungi degraded solid lignite coal to a black liquid product which never appeared in cultures unless fungi and coal were present together. Apparently, lignite coal can serve as the principal substrate for the growth of the fungi. Infrared analyses of the liquid products of lignite degradation showed both similarities to and differences from the original lignite.

Although coal conversion technology has been studied since at least 1780 (9), little attention has been directed toward the biological degradation of coal, perhaps because there have been few reports of organisms which can metabolize coal. Belly and Brock (4) developed a technique for measuring $^{14}CO_2$ uptake by chemolithotropic bacteria directly in pyritic materials associated with coal and coal refuse. Maximal $^{14}CO_2$ uptake occurred in coal refuse material 2 to 3 years old. Only slight incorporation was reported for fresh material or material 40 years old. Kucher and Turovskii (13) reported optimal conditions for the growth of the yeast *Candida tropicalis* in aqueous extracts of oxidized coal. Holladay et al. (10) reported the biodegradation of phenolic waste liquors resulting from the pyrolysis of coal.

We found no reports of fungi that grow directly on and metabolize naturally occurring coal. This is the first report of fungal growth either directly on lignite coal or in minimal medium supplemented with crushed coal.

MATERIALS AND METHODS

Polyporus versicolor ATCC 12679 and *Poria monticola* ATCC 11538 were obtained from the American Type Culture Collection, Rockville, Md. Lignite coal was obtained from the American Colloid Co., Skranton, N. Dak. A geological analysis of the lignite indicated that it originated in the upper Paleocene era. All coal used in the experiments was derived from bulk pieces in which the grain of the woody tissues from which it was formed was preserved.

Both *Polyporus versicolor* and *Poria monticola* were routinely maintained and cultured in Sabouraud maltose broth and on Sabouraud maltose agar (28°C; 80% relative humidity; pH 5.8). All culture media were sterilized in 1-pint (ca. 0.473-liter) jars before inoculation, all fungal transfers were performed aseptically in a laminar flow hood, and all materials which came into contact with the cultures were sterilized before use. Experimental cultures were incubated as described for stock cultures. Two-week-old 1-pint broth cultures of both *Polyporus* and *Poria* mycelia were aseptically accumulated and placed into 100-ml milk dilution bottles containing sterile glass beads and distilled and deionized (D/D) water. The bottles were then stoppered and shaken to disrupt hyphae. Accumulated hyphal suspensions were then filtered through ground-glass filter paper and washed with 1 liter of D/D water to eliminate residual broth media. Approximately 0.5 g (wet weight) of mycelium was then transferred to Noble agar medium (1% [wt/vol] Noble agar, D/D water) and lignite agar medium (1% [wt/vol] Noble agar, 7% [wt/vol] powdered lignite, D/D water; lignite powder was added to the warm dissolved agar, and the pH was adjusted to 5.8 with 0.5 N KOH). Before inoculating crushed lignite substrate medium or solid lignite substrate medium, the hyphae were filtered as described above, and 0.5-g samples were suspended in 1 ml of D/D water. To prepare crushed lignite substrate medium, powdered lignite was filtered through an 80/100-mesh screen and hydrated with 0.1 N KOH to 130% of its water-holding capacity (1); the pH was adjusted to 5.8. Solid lignite substrate medium was prepared as follows. Sabouraud maltose agar (3 to 6%, wt/vol) was inoculated with 1-ml samples of the hyphal suspension described above, and the hyphae were spread over the surface with a sterile glass rod. These cultures were then incubated for 5 days. A sterilized polypropylene grid was placed on top of each culture, and a small (2-cm^3) sterile piece of solid lignite coal was placed on top of the grid to isolate the coal from contact with the agar. The cultures were then incubated for 28 days.

Infrared (IR) analyses of materials were done on a Perkin-Elmer infrared double-beam grating spectrophotometer (model 283). Crushed lignite was analyzed

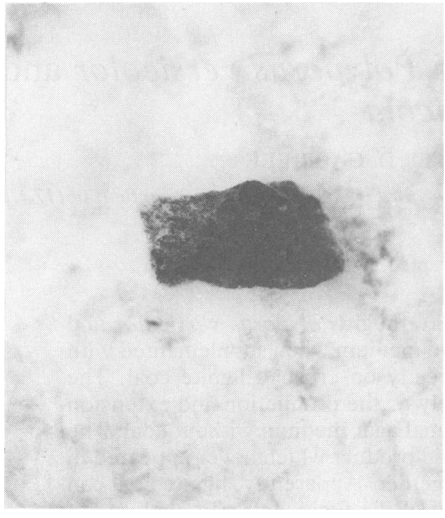

FIG. 1. Early stage of growth of *Polyporus* hyphae onto a piece of solid lignite coal 10 days after the lignite piece was placed onto a polypropylene grid which separates the lignite from the surface of the culture medium.

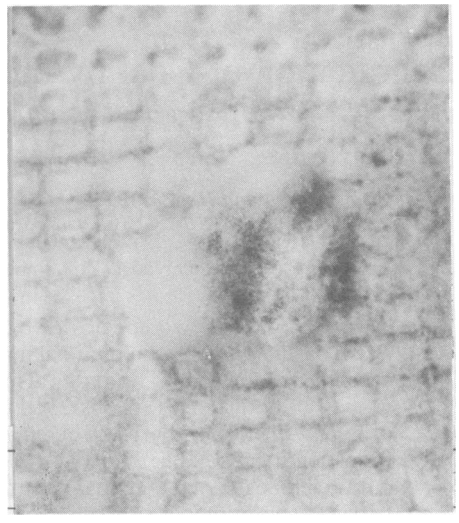

FIG. 2. Growth of *Polyporus* hyphae onto a piece of solid lignite coal 28 days after the lignite piece was placed onto a polypropylene grid which separates the lignite from the surface of the culture medium. Note that the hyphae have completely covered the lignite piece.

as a mugol mull cast film on both sodium chloride and silver chloride window materials. A hexachloro-1,3-butadiene mull was prepared for the hydrocarbon regions 3,000 to 2,800, 1,500 to 1,300, and 750 to 650 cm^{-1}. IR analyses of liquid degradation products were performed as cast films on sodium chloride and silver chloride window materials. Cast films were both air and oven dried (120°F; 60 min) before running the spectra. No differences were observed between these preparations.

RESULTS

Rapid growth of both *Polyporus versicolor* and *Poria monticola*, measured qualitatively as the production and extension of hyphae, occurred on the Sabouraud malt agar used routinely to maintain the fungi. The agar in these continuous cultures always maintained the original straw color.

The growth of *Polyporus versicolor* also occurred in lignite agar medium and on crushed lignite coal. Hyphal growth was more extensive and luxurious on lignite agar than on crushed lignite substrate. The production and growth of *Poria* hyphae were less extensive than those of *Polyporus* hyphae in identical cultures of lignite agar. No fungal growth was observed in Noble agar cultures, and the agar maintained the original pale straw color. The agar may be a source of micronutrients which enhance fungal growth, but Noble agar alone cannot maintain the fungi or allow their growth. We interpreted this to mean that the principal substrate for the growth of both *Polyporus versicolor* and *Poria monticola* in lignite agar and on crushed lignite substrate was the lignite coal. In the solid lignite cultures, vegetative hyphae of both fungi were observed to grow up onto the solid lignite piece and completely cover it (Fig. 1 and 2). The grain of the plant material from which the lignite was formed could be seen on the pieces, and the hyphae followed the pattern of the grain. In approximately half of all cultures, several drops of a black viscous liquid substance appeared on top of the hypha-covered lignite pieces (Fig. 3 and 4). After some of this liquid was drawn off for analysis, several more milliliters of black liquid were produced. The production appeared to be continuous because the Sabouraud malt agar, initially a pale straw color, progressively darkened and became uniformly black during the incubation period after the fungal hyphae covered the lignite piece. In identical cultures incubated as described above but without inoculation with *Polyporus versicolor* or *Poria monticola* and in Sabouraud maltose agar cultures of the fungi, the agar maintained a pale straw color and never darkened during the time of testing (Fig. 5). This established that the source of the black liquid was the lignite coal and that the digestion of the coal by *Polyporus versicolor* or *Poria monticola* was necessary to produce the black liquid digestion product.

Both lignite and the black liquid exudates were analyzed by IR spectroscopy. The absorption spectra are shown in Fig. 6. Figure 6A shows the IR absorption spectrum of a mugol mull of similar lignite coal samples from which

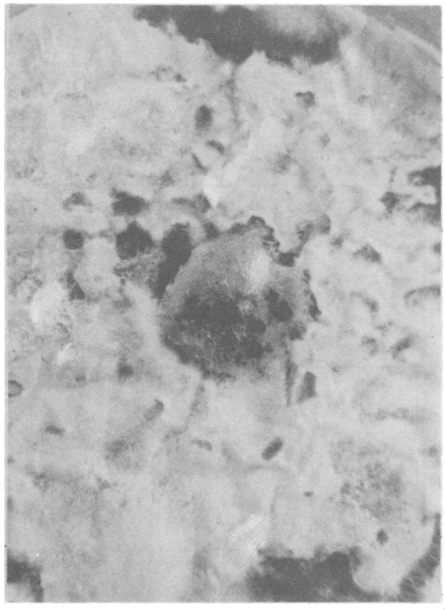

FIG. 3. Production by *Polyporus versicolor* of a black liquid digestion product from a solid piece of lignite treated as described in the legend to Fig. 2. The black liquid appears as several drops on top of the hypha-covered lignite piece and has also diffused into the medium, causing it to darken.

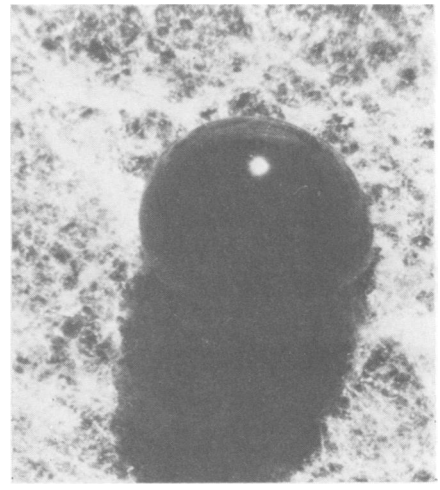

FIG. 4. Close-up of a drop of the black liquid digestion product produced as described in the legend to Fig. 3 on the surface of the lignite piece. Note the arrangement of fungal hyphae on the lignite and the spherical nature of the liquid product.

the black liquid digestion product was produced. The band at 3,400 cm^{-1} (H-bonded OH) is broad due to extensive hydrogen bonding (7). In lignite, the bands at 2,920 and 2,850 cm^{-1} (aliphatic C—H stretching) do not appear to indicate a high degree of aliphatic hydrocarbon structure (4, 8). This is typical of lignite coal. The shape and intensity of the band at 1,600 cm^{-1} are typical of lignite, and a controversy over specific band assignment has been reported (8). The shoulder at 1,700 cm^{-1} and the band at 1,600 cm^{-1} combined probably represent high concentrations of either conjugated aromatic structures or chelated and conjugated carbonyl structures, such as acetylacetone, kojic acid, or hydroxyacetophenes, or both (3, 8). The remaining regions of complex absorptions from 1,300 to 900 cm^{-1} represent combinations of phenoxy and ether linkages as well as clay minerals (1,040 to 910 cm^{-1}) (7). The lack of absorption peaks at 720 to 800 cm^{-1} indicates that no hydrocarbon chains longer than C_4 were present (3).

Figures 6B and C show IR absorption spectra of the black viscous liquid exudates produced from fungus-covered lignite pieces in cultures with *Polyporus versicolor* and *Poria monticola*, respectively. Both spectra show similar absorption patterns in the regions of 3,400 and 1,600 cm^{-1}. These also represent the principal functional group absorptions of lignite (4, 7, 8). The spectrum in Fig. 6B also shows functional group absorptions at 3,200 cm^{-1} (N—H) (14), 1,720 cm^{-1} (C—O) (3), 1,680 cm^{-1} (aryl ketones or conjugated carbonyl) (5), and 1,410 cm^{-1} (carboxylic anionic groups, carbonate, and CH_2) (4).

The spectra in Fig. 6B and C also show a shift of the band at 1,600 cm^{-1} present in lignite down to 1,580 cm^{-1}. Band shifts to lower wave numbers generally represent an increase in the resonance of functional groups (8). This same shift of absorption has been reported to occur in the IR absorption spectrum of lignite after pyrolysis at 450°C (6). We interpreted this result to mean that conjugated aromatic rings responsible for absorption at 1,600 cm^{-1} had been structurally modified by the digestion of lignite by *Polyporus versicolor* and *Poria monticola* at 28°C in a manner similar to the ring modification produced by pyrolysis at 450°C.

The spectrum in Fig. 6C shows fewer regions of IR absorption compared with the spectrum in Fig. 6B; this indicates the presence of fewer functional groups.

DISCUSSION

Lignite coals originate from the compaction and induration of the altered remains of diverse plants, all of which contain lignin in various amounts. During the coalification process, lignin-like structures are preserved and may predominate over other woody tissue remains. Buravis et al. (6) reported that the chemical analysis of young lignites showed that they contain from 35 to 70% lignin-like compounds and that older lignites are likely to contain compounds formed

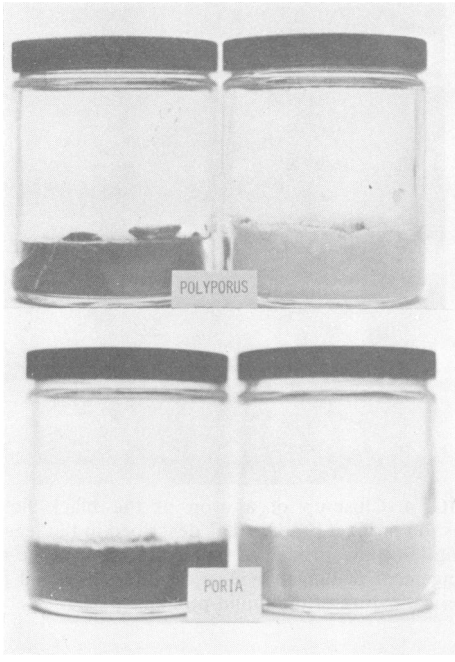

FIG. 5. Darkening of the Sabouraud maltose agar culture medium by *Polyporus versicolor* (top left) and *Poria monticola* (bottom left) after the production of the black liquid digestion product from lignite. In identical cultures incubated as described in the text but without the coal, the medium remained a pale straw color (right).

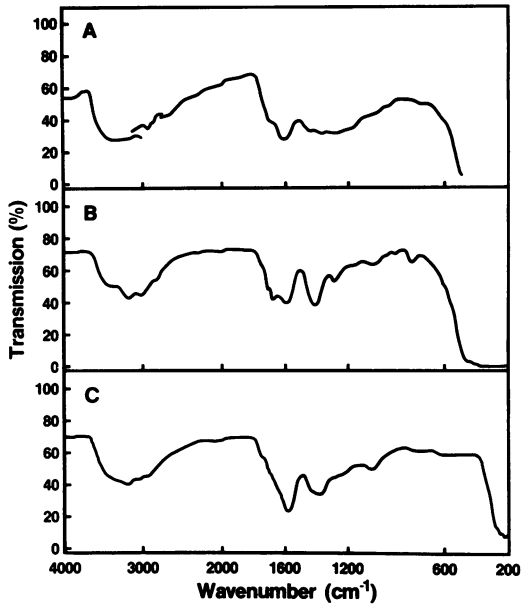

FIG. 6. IR absorption spectra of a mugol mull of lignite (A) and the black liquid digestion products produced from lignite by *Polyporus versicolor* (B) and *Poria monticola* (C). Note the shift of the characteristic band at 1,600 cm^{-1} of lignite to bands at 1,590 cm^{-1} in both of the liquid digestion products.

by the condensation of these structures. Thus, lignin and lignite share a common origin, each being derived from plant tissue.

Polyporus versicolor and *Poria monticola* are wood-decaying organisms with two different mechanisms of digestive action. *Polyporus versicolor* decomposes lignified tissues by digesting polyphenylpropane polymers and associated aromatic ring structures of the lignin polymer. This white-rot decay is reported to be based on the production of polyphenoloxidase and peroxidase enzymes (15, 16). Ander et al. (2) have reported that a phenol oxidaseless mutant of the white-rot fungus *Sporotrichum pulverulentum* lost the ability to degrade lignin and other wood components. The enzyme cellobiose:quinone oxidoreductase has been reported in cultures of *Polyporus versicolor* and seems to be important in lignin degradation (2). However, the mechanism and biochemical pathways of lignin biodegradation have not been specifically defined (12). The polymeric structures derived from lignin and present in lignites might serve as suitable substrates for digestion by *Polyporus versicolor*.

Poria monticola decomposes wood by digesting polysaccharides, producing only a small loss of lignin. This brown-rot decay has been reported to be based on the production of β-glucosidases which attack principally celluloses (11). Recently, Schmidt et al. (17) have proposed that the brown-rot mechanism occurs nonenzymatically through the production of free radicals which degrade cellulosic polymers. This physiological difference could explain why the growth of *Polyporus versicolor* was more extensive and rapid than that of *Poria monticola* on lignite agar medium.

We have not established that the degradation of lignite occurred enzymatically or that specific digestive enzymes were produced. Although some activity of fungal growth was necessary to degrade the lignite, the biochemical mechanism has not been defined.

The fact that only half of all cultures on lignite pieces produced the black liquid digestion product may indicate that a process of adaptation to lignite has occurred in these cultures or that the lignite sample has a heterogeneous chemical composition. Due to differences in the digestive action of the two fungi, variations in the chemical composition of the black liquid breakdown products of lignite would be expected; this was confirmed by IR analyses. The products produced from lignite by *Polyporus versicolor* and *Poria monticola* have similarities in basic composition to lignite and to each other, but they appear to be different.

LITERATURE CITED

1. **American Society for Testing and Materials.** 1976. Standard method of testing wood preservatives by laboratory soilblock cultures. ASTM designation D1413-76. American Society for Testing and Materials, Philadelphia, Pa.
2. **Ander, P., A. Hatakka, and K. E. Eriksson.** 1980. Degradation of lignin and lignin-related substances by *Sporotrichum pulverulentum* (*Phanerochaete chrysosporium*), p. 1–15. *In* T. Kirk, T. Higuchi, and H. Chang (ed.), Lignin biodegradation: microbiology, chemistry, and potential applications, vol. 2. CRC Press, Boca Raton, Fla.
3. **Bellamy, L. J.** 1975. The infra-red spectra of complex molecules, p. 13–26, 149–202, 231–262, 277–291. Chapman and Hall, London.
4. **Belly R. T., and T. D. Brock.** 1974. Ecology of iron-oxidizing bacteria in pyritic materials associated with coal. J. Bacteriol. **117**:726–732.
5. **Berkowitz, N.** 1979. An introduction to coal technology, p. 121. Academic Press, Inc., New York.
6. **Buravis, S., A. F. Gaines, T. Hasadrsi, A. Prasertwitayakij, and N. Sucharitakul.** 1970. The infra-red spectra of tertiary lignites. Fuel **49**:180–187.
7. **Friedel, R. A.** 1976. Infra-red in coal structure research, p. 312–341. *In* D. N. Kendall (ed.), Applied infrared spectroscopy. Reinhold Publishing Corp., New York.
8. **Fujii, Y., Y. Osawa, and H. Sugimura.** 1970. Infrared spectra of Japanese coal: the absorption bands at 3030, 2920, and 1600 cm^{-1}. Fuel **49**:68–75.
9. **Gorbaty, M., F. Wright, R. Lyon, R. Schlosberg, Z. Baset, R. Liotta, B. Silbernagel, and D. Neskora.** 1979. Coal science: basic research opportunities. Science **206**:1029–1034.
10. **Holladay, D. W., C. W. Hancher, C. D. Scott, and D. D. Chilcote.** 1978. Biodegradation of phenolic waste liquors in stirred-tank, packed bed, and fluidized bed reactors. J. Water Pollut. Control Fed. **50**:2573–2589.
11. **Kirk, T. K.** 1973. Chemistry and biochemistry of decay, p. 141–181. *In* D. Nicholas (ed.), Wood deterioration and its prevention by preservative treatments. Syracuse University Press, Syracuse, N.Y.
12. **Kirk, T. K.** 1980. Lignin biodegradation: summary and perspectives, p. 235–243. *In* T. K. Kirk, T. Higuchi, and H. Chang (ed.), Lignin biodegradation: microbiology, chemistry, and potential applications, vol. 2. CRC Press, Boca Raton, Fla.
13. **Kucher, R. V., and A. A. Turovskii.** 1977. Cultivation of *Candida tropicalis* on coal substrates. Mikrobiologiya **46**:583–585.
14. **Martin, F.** 1975. Humic acids from lignite. 1. Analytical characteristics and thermal degradation. Fuel **54**:236–240.
15. **Mason, H. S.** 1955. Comparative biochemistry of the phenolase complex. Adv. Enzymol. Relat. Subj. Biochem. **16**:105–184.
16. **Oglesby, R. T., R. F. Christman, and C. H. Diver.** 1968. The biotransformation of lignin to humus—facts and postulates. Adv. Appl. Microbiol. **9**:171–184.
17. **Schmidt, C. J., B. Whitten, and D. Nicholas.** 1981. A proposed role for oxalic acid in non-enzymatic wood decay by brown-rot fungi. Proc. Am. Wood Preserv. Assoc. **77**:1–7.