APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1983, p. 1201–1206
0099-2240/83/111201-06$02.00/0

Vol. 46, No. 5

Isolation of a Bacterium Capable of Degrading Peanut Hull Lignin

THOMAS J. KERR,[1]* ROBERT D. KERR,[2] AND RONALD BENNER[1]

Department of Microbiology, University of Georgia, Athens, Georgia 30602,[1] and R. D. Kerr Co., Salem, Alabama 36874[2]

Received 22 July 1983/Accepted 2 September 1983

Thirty-seven bacterial strains capable of degrading peanut hull lignin were isolated by using four types of lignin preparations and hot-water-extracted peanut hulls. One of the isolates, tentatively identified as *Arthrobacter* sp., was capable of utilizing all four lignin preparations as well as extracted peanut hulls as a sole source of carbon. The bacterium was also capable of degrading specifically labeled [^{14}C]lignin-labeled lignocellulose and [^{14}C]cellulose-labeled lignocellulose from the cordgrass *Spartina alterniflora* and could also degrade [^{14}C]Kraft lignin from slash pine. After 10 days of incubation with [^{14}C]cellulose-labeled lignocellulose or [^{14}C]lignin-labeled lignocellulose from *S. alterniflora*, the bacterium mineralized 6.5% of the polysaccharide component and 2.9% of the lignin component.

During the past few years, increased attention has been given to the role of bacteria in the decomposition of lignin and lignocellulosic plant materials. Wood rot fungi have been considered to be the primary decomposers of lignin in nature. However, the ability of several bacterial strains to degrade chemically isolated lignin preparations as well as radiolabeled natural and synthetic lignins has been established.

Cartwright and Holdom (4) isolated an *Arthrobacter* strain capable of utilizing enzymic lignin (lignin released by a *Cellulomonas* spp.) as a sole carbon source. Odier et al. (18) reported the isolation of 11 gram-negative bacteria, identified as *Pseudomonas*, *Xanthomonas*, and *Acinetobacter* species, that were capable of assimilating poplar dioxane lignin without a cosubstrate. Several *Nocardia* strains have been shown to decompose radiolabeled natural and synthetic lignins and to utilize lignin as a carbon source (12, 13, 24). A *Bacillus* strain (21) and several *Streptomyces* strains have been shown to degrade radiolabeled natural lignins (6, 7, 10, 19, 23). Thus, it is now clear that bacteria are capable of extensive degradation of all structural components of lignin.

Peanut hulls contain more lignin (approximately 32%) than most hardwoods and softwoods, and they are very resistant to biodegradation (1; T. J. Kerr, R. Benner, W. E. Rigsby, and J. H. Woodward, unpublished data). We report the isolation of 37 bacterial strains capable of degrading and assimilating at least one of four types of lignin preparations which were chemically isolated from peanut hulls. Of the 37 isolates, only 1, tentatively identified as *Arthrobacter* sp., was capable of utilizing all four lignin preparations as a sole source of carbon. The ability of this bacterium to degrade specifically labeled [^{14}C]lignin-labeled lignocellulose and [^{14}C]cellulose-labeled lignocellulose from the smooth cordgrass *Spartina alterniflora* and [^{14}C]Kraft lignin from slash pine was also investigated.

MATERIALS AND METHODS

Lignin preparations. Peanut hulls were supplied by the Columbian Peanut Company of Ozark, Ala., and Stevens Industries, Dawson, Ga. The peanut, *Arachis hypogaea*, was of the Florunner variety and was hammer milled to 0.25 in. (ca. 6 mm) in size. Before use as a growth substrate, peanut hulls were extracted with boiling water for 1 h (four changes of water during extraction) and dried for 24 h at 70°C. Lignin was isolated from peanut hulls by four different procedures.

(i) Dioxane lignin. Hammer-milled peanut hulls (0.25 in.) were extracted in a Soxhlet extractor for 50 h with boiling ethanol-benzene (1:1) and were dried in a vacuum desiccator. Extractive-free hulls were then extracted for 12 h at 90°C with dioxane-water (9:1) containing the equivalent of 0.2 N HCl. The extract was concentrated under a vacuum, and the lignin was precipitated in deionized distilled water. The precipitated lignin was washed with water, dried, and washed with petroleum ether (2).

(ii) Milled wood lignin. Hammer-milled peanut hulls (0.25 in.) were extracted with boiling ethanol-benzene (1:2) for 48 h and then with 95% ethanol for 24 h. Extractive-free hulls were ball milled for 9 days. Milled hulls were extracted with dioxane-water (9:1) for 12 h at 90°C. The solvent was evaporated under a vacuum, and the residue was dissolved in aqueous acetic acid (9:1). The acidified solution was added

dropwise to water, and the precipitate was collected by centrifugation. The precipitated lignin was thoroughly washed with petroleum ether (2).

(iii) Klason lignin. Extractive-free hulls (as above) were treated with 72% sulfuric acid at 15°C for 2 h. The mixture was diluted with water to a 3% acid concentration and refluxed for 4 h. The residue was washed thoroughly with water (2).

(iv) Hydrochloric acid lignin. Hammer-milled hulls (0.25 in.) were treated with hydrochloric acid (specific gravity, 1.19 at 5°C) at 5°C for 2 h with shaking. The temperature was allowed to rise to room temperature. Ice was added to the mixture, which was allowed to stand at room temperature for 18 h. Water was added, and the lignin was collected on a filter. The precipitate was washed and boiled in water with the gradual addition of sodium carbonate until the mixture reached neutrality. The lignin was collected by filtration (2).

Lignin and peanut hulls (extracted with boiling water) were used at a concentration of 0.5 g/liter in both solid and liquid media. Hulls used in liquid media were ball milled to pass through a 0.1-mm sieve. Lignins and hulls used in solidified media were dissolved in 0.25 N NaOH (0.5 g of hulls per 10 ml of NaOH), filter sterilized, and added to sterile media containing 7 g of K_2HPO_4, 3 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, and 0.1 g of $MgSO_4 \cdot 7H_2O$ per liter of tap water (4). Hot-water-extracted peanut hulls were only partially soluble (14.1%, dry weight) in 0.25 N NaOH. An equivalent amount of sterile 0.25 N HCl was added to neutralize the media.

Preparation of ^{14}C-labeled lignocelluloses and [^{14}C]Kraft lignin. The smooth cordgrass *S. alterniflora* was specifically labeled in the lignin or polysaccharide component by placing the cut stems of individual plants in 1 ml of a sterile aqueous solution containing 5 μCi of L-[*U*-^{14}C]phenylalanine or D-[*U*-^{14}C]glucose (8, 17). Additional sterile water was added to keep the cut ends immersed during a 72-h incubation period. The labeled material was dried (55°C) and ground to pass through a 40-mesh screen. The plant material was then serially extracted in boiling ethanol, ethanol-benzene (1:2), and water (17). Extractive-free lignocellulose was collected on a glass fiber filter, washed with ethanol, and dried at 55°C. The ^{14}C-labeled lignocelluloses were characterized for the distribution of label between the lignin and polysaccharide components by Klason hydrolysis as previously described (17). Samples of ^{14}C-labeled lignocellulose were also digested in the protease pepsin to determine the percentage of radiolabel possibly associated with protein (17). The specific activities of ^{14}C-labeled lignocelluloses were determined by combusting 10-mg samples in a biological oxidizer (model OX300; R. J. Harvey Co., Hillsdale, N.J.) and trapping the released $^{14}CO_2$ in a liquid scintillation medium (17).

The [^{14}C]lignin-labeled *Spartina* lignocellulose had a specific activity of 23,238 dpm/mg, 70.9% of the label was recovered in the Klason lignin fraction, and 19.0% of the label was solubilized during pepsin digestion. The polysaccharide-labeled *Spartina* lignocellulose had a specific activity of 6,889 dpm/mg, 61.9% of the label was recovered in the acid-soluble fraction, and 21.9% of the label was solubilized during pepsin digestion.

Wood from the slash pine, *Pinus elliottii*, was labeled in the lignin component by the methods described above for labeling *S. alterniflora*. The [^{14}C]lignin-labeled pine lignocellulose had a specific activity of 2,825 dpm/mg, 90% of the label was recovered in the Klason lignin fraction, and 2.8% of the label was solubilized during pepsin digestion. After extracting the wood to remove unincorporated label, the [^{14}C]lignin-labeled lignocellulose was pulped with a laboratory-scale Kraft pulping system (5, 9, 16). The ^{14}C-labeled lignocellulose was heated to 160°C for 4 h in Kraft pulping liquor (1:25 M NaOH, 0.25 M Na_2S). After cooling, the mixture was filtered and [^{14}C]Kraft lignin was precipitated from the pulping liquor by acidification with H_2SO_4 to pH 2. The precipitated lignin was collected by centrifugation and washed three times with deionized water. The specific activity of the [^{14}C]Kraft lignin was 2,904 dpm/mg.

Isolation of lignin-degrading bacteria. Decaying peanut hulls were obtained from the bottom of a peanut hull pile situated on a farm in Salem, Ala. The hulls had been weathered in the open for approximately 5 years and showed signs of physical degradation. Five 250-ml flasks were prepared, each containing 100 ml of basal salts solution (as above) and 50 mg of water-extracted hulls or one of the four lignins. Approximately 10 ml of decaying hulls was placed in 100 ml of sterile saline and shaken for 5 min. One-milliliter samples were used to inoculate the five 250-ml flasks. The flasks were incubated at 26°C for 24 h in a shaking incubator, and then 0.1-ml samples were used to inoculate agar plates containing basal salts and hulls or one of the lignin preparations. Plates were incubated for 24 h at 26°C. Each colony was picked and streaked on five agar plates containing basal salts medium and one of the five substrates (hulls or lignins) used as carbon sources. The bacterium that grew on all five carbon sources was tested for various biochemical reactions and for sensitivity to dyes and antibiotics as described by Kerr (15).

Degradation of ^{14}C-labeled lignocelluloses and [^{14}C]Kraft lignin. The bacterial isolate (KB-1) capable of growing on all four lignin preparations as well as on extracted hulls was grown overnight (shaking, 26°C) in a basal salts solution containing *Spartina* lignocellulose. After permitting the larger lignocellulose particles to settle, 20-ml portions of the culture were added to 125-ml milk dilution bottles (equipped with gassing ports) containing 10 mg of [^{14}C]lignin-labeled lignocellulose from *S. alterniflora*, [^{14}C]cellulose-labeled lignocellulose from *S. alterniflora*, or [^{14}C]Kraft lignin from slash pine. To investigate the effect of an added carbon source on [^{14}C]Kraft lignin degradation, 20 mg of alpha-cellulose was added to one set of bottles. Bottles were incubated in duplicate in the dark at 30°C with shaking (125 rpm). Controls were killed with 5% Formalin and did not evolve any $^{14}CO_2$ during incubation with isolate KB-1. Mineralization of the radiolabeled substrates was monitored every 48 h by flushing the bottles with CO_2-free sterile air and trapping the evolved $^{14}CO_2$ in a series of two scintillation vials containing liquid scintillation counting medium (17). Water-soluble radiolabel present in the incubations containing ^{14}C-labeled lignocellulose from *S. alterniflora* was quantified by filtering the contents of the bottles through Nuclepore filters (Nuclepore Corp., Pleasanton, Calif.; pore size, 1.0 μm), acidifying the filtrate (pH < 2) to remove $^{14}CO_2$, and assaying 1-ml portions for radioactivity.

Scanning electron microscopy. For scanning electron microscopy, a 48-h (nutrient agar) culture of the bacterial isolate KB-1 was suspended in sterile saline and filtered onto a Nuclepore filter. The filter was immediately placed in a 3% glutaraldehyde–1% osmium tetroxide solution in 50 mM cacodylate buffer (pH 7.4) and fixed for 45 min at 4°C. The fixed cells were then washed in a 0.1 M cacodylate buffer with 5% sucrose. After dehydration in a graded ethanol series, the cells were critical point dried, sputter coated with gold-palladium, and examined in a Cambridge Stereoscan mark IIA scanning electron microscope at 20 kV.

Chemicals and radioisotopes. All chemicals utilized in the treatment, extraction, and analysis of peanut hulls were reagent grade and were obtained from either Fisher Scientific Co., Atlanta, Ga., or Sigma Chemical Co., St. Louis, Mo. Radioisotopes, D-[*U*-^{14}C]glucose, and L-[*U*-^{14}C]phenylalanine were obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

Thirty-seven bacteria were isolated from decaying peanut hulls by the use of water-extracted hulls and four different lignin preparations isolated from peanut hulls (Table 1). Of this number, 8 were isolated from media containing water-extracted hulls (designated P strains), 9 from media containing Klason lignin (designated K), 13 from media containing hydrochloric acid lignin (H), 4 from media containing dioxane lignin (D), and 3 from media containing milled wood lignin (M). A majority of the isolates (25) grew on the NaOH extract of water-extracted hulls, and 23 isolates grew on the milled wood lignin preparation. Only eight of the isolates grew on dioxane lignin, but all of the isolates from the liquid dioxane lignin culture grew on the NaOH extract of water-extracted peanut hulls, and three out of four grew on milled wood lignin. It is also interesting to note that very few of the isolates grew on Klason lignin, except for bacteria originally isolated from liquid Klason lignin cultures.

Although 16 isolates grew on three of the five preparations, only 1 bacterial isolate was capable of utilizing all five materials as a sole carbon source. The bacterium capable of growing on all five preparations was K-7. This isolate was redesignated KB-1 to indicate that it was the only isolate capable of utilizing all five preparations as a carbon source. When inoculated onto agar plates containing basal salts and no added carbon source, KB-1 did not grow.

When grown on nutrient agar at 26°C, strain KB-1 appeared as a gram-positive, short, stubby rod (Fig. 1) that possessed a sporelike vacuole situated in the middle of the cell when viewed under a light microscope. This vacuole did not retain the Gram stain and thus appeared to be hollow. When KB-1 was subjected to a spore stain, it failed to show any sign of a spore. When grown in nutrient broth at 26°C in a shaking incubator for 24 h and Gram stained, KB-1 appeared as small, gram-negative cocci with a few (less than 1%) larger cocci that were gram positive.

Biochemical testing of KB-1 indicates that it can use a number of carbohydrates as carbon sources, including glucose, maltose, xylose, mannose, and sucrose, but it is not capable of using arabinose (Table 2). This is very interesting because arabinose is one of the primary carbohydrates found in peanut hulls (20). The bacterium is catalase positive and NO_2 positive,

TABLE 1. Growth of bacterial isolates on minimal media with various lignin preparations[a]

Bacterial isolate	Peanut hull[b]	Klason lignin	HCl lignin	Dioxane lignin	Milled wood lignin
P-1	+	−	+	−	+
P-2	+	−	−	−	+
P-4	+	+	−	−	−
P-6	+	−	−	−	+
P-8	+	−	+	+	−
P-9	+	−	−	−	+
P-10	+	−	−	−	+
P-13	+	−	−	−	+
K-1	+	+	−	−	+
K-3	+	+	−	−	+
K-4	−	−	−	−	+
K-5	+	+	−	−	−
K-6	−	+	−	−	−
K-7	+	+	+	+	+
K-8	−	+	−	−	−
K-10	+	+	+	−	−
K-11	−	+	−	−	+
H-1	+	−	+	−	+
H-2	−	+	+	+	−
H-3	+	−	+	−	+
H-4	+	−	+	−	+
H-5	−	+	+	+	−
H-6	−	+	+	+	−
H-7	−	−	+	−	+
H-8	−	−	+	−	−
H-11	+	−	+	−	+
H-14	−	−	+	−	−
H-15	+	−	+	−	+
H-16	−	−	+	−	−
H-17	−	−	+	−	−
D-1	+	−	−	+	+
D-2	+	−	−	+	+
D-3	+	−	+	−	−
D-4	+	−	−	+	+
M-1	+	−	−	−	+
M-3	+	−	−	−	+
M-4	+	−	−	−	+

[a] Twelve isolates did not grow upon transfer to fresh media and they are omitted from the table.

[b] NaOH extract of hot-water-extracted peanut hulls.

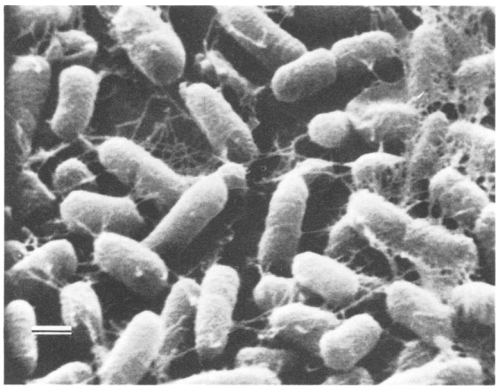

FIG. 1. Gram-positive rods of bacterial isolate KB-1 when grown on nutrient agar. Bar, 1 μm.

and it utilizes citrate, which are three of the main biochemical tests used in the identification of *Arthrobacter* spp. Although KB-1 is gram positive during a portion of its life cycle, it is not susceptible to penicillin or ampicillin but is extremely susceptible to chloramphenicol, sulfathiazole, tetracycline, and kanamycin.

Degradation of radiolabeled substrates by KB-1. After 10 days of incubation, strain KB-1 mineralized 2.9% of the lignin component and 6.5% of the polysaccharide component of *S. alterniflora* ^{14}C-labeled lignocellulose (Fig. 2). The bacterium degraded the lignin and cellulosic portions of the lignocellulose simultaneously. Rates of degradation were most rapid during the first 6 days of incubation. At the end of the incubation, filtrates were analyzed for ^{14}C-labeled water-soluble degradation products. Bottles containing [^{14}C]-labeled lignin lignocellulose had an average of 3.2% of the added label in soluble form. The Formalin-killed control contained 0.8% of added label in soluble form. Bottles containing [^{14}C]cellulose-labeled lignocellulose had an average of 3.7% of the added label in soluble form. The Formalin-killed control contained 2.9% of added label in soluble form.

The [^{14}C]Kraft lignin was mineralized at 30% of the mineralization rate of [^{14}C]lignin-labeled lignocellulose from *S. alterniflora* (Fig. 2). The lignin portion of *Spartina* lignocellulose has been reported to be mineralized approximately 4 to 6 times faster than Kraft lignin by natural microbial populations found in salt marsh sediments (14, 16). In the presence of added cellulose, KB-1 mineralized [^{14}C]Kraft lignin at 67% of the rate in the absence of added cellulose.

DISCUSSION

By utilizing four types of lignin preparations isolated from peanut hulls in addition to water-extracted hulls, we were able to isolate 37 bacteria capable of using lignin preparations as sole carbon and energy sources. Only 1 (KB-1) of the 37 isolates was capable of growing on all five materials. Although the data indicate that the bacterium utilizes polysaccharides in addition to lignin, good growth of KB-1 was obtained by using lignin preparations with no added carbohydrates or polysaccharides.

TABLE 2. Biochemical reactions, dye sensitivity, and antibiotic susceptibility of isolate KB-1

Test	Result	Test	Result	Test	Result
Biochemical		Inositol	−	Dye sensitivity	
Arginine dihydrolase	−	Sorbitol	−	Malachite green	+
Lysine decarboxylase	−	Rhamnose	−	Crystal violet	+
Ornithine decarboxylase	−	Melibiose	−	Methyl green	+
Citrate	+	Amygdalin	−		
Urease	−	Arabinose	−	Antibiotic susceptibility[a]	
Tryptophan deaminase	−	Motility	−	Streptomycin	++
Indole	−	Fat hydrolysis	−	Penicillin	−
Voges-Proskauer	+	DNAse activity	−	Rifampin	−
Gelatin	−	Starch hydrolysis	−	Bacitracin	−
Oxidase	−	NO_2	+	Erythromycin	−
Catalase	+	N_2 ↑	+	Ampicillin	−
Methyl red	−	Blood agar	Gamma	Tetracycline	++
Maltose	+ Gas			Chloramphenicol	++++
Glucose	+	Triple sugar iron		Sulfathiazole	+++
Xylose	+ Gas	Slant	Acid	Kanamycin	++
Mannose	+ Gas	Base	Acid	Novobiocin	+
Sucrose	+	H_2S	−	Polymyxin B	+
Lactose	−	Gas	+	Neomycin	+
Mannitol	−			Vancomycin	−

[a] Results of antibiotic susceptibility tests: +, 5-mm clearing; ++, 10-mm clearing; +++, 15-mm clearing; ++++, 20-mm clearing.

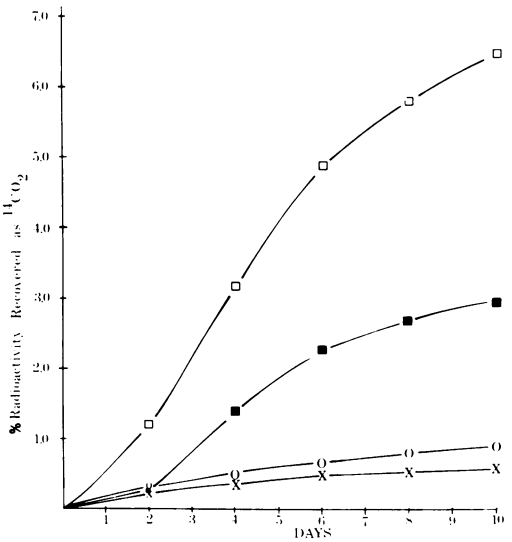

FIG. 2. Mineralization by bacterial isolate KB-1 of [^{14}C]lignin-labeled lignocellulose (■) and [^{14}C]cellulose-labeled lignocellulose (□) from *S. alterniflora* and of [^{14}C]Kraft lignin from *P. elliottii* with (X) and without (O) 20 mg of added cellulose (1 mg/ml). Each point represents the mean of two replicate experiments.

The tentative identification of the bacterium as an *Arthrobacter* species is based on its pleomorphic characteristics, even though they appear to be reversed. Usually, *Arthrobacter* spp. are isolated as cocci, and when put into fresh media they become rod shaped, then revert to cocci in older media. The bacterium KB-1 was isolated as a rod and grows as a rod in solid media, only changing to the coccoid form in liquid media. In addition to this pleomorphism, results from the biochemical tests generally match those described for *Arthrobacter* species in Bergey's manual (3).

The only other report of a lignolytic *Arthrobacter* sp. was by Cartwright and Holdom (4), who reported the isolation and characterization of an *Arthrobacter* sp. that utilized enzymic lignin (from birch) as a sole carbon source. Because they failed to isolate a number of different organisms capable of growing on this enzymic lignin, they concluded that bacteria as a whole have no major role in the degradation of lignin as it occurs in nature. This broad generalization does not seem justified. From our experience, several methods and substrates should be used to isolate lignin-degrading bacteria. Bacteria appear to be capable of significant degradation of peanut hull lignin and lignocellulose.

Several of the lignin preparations (Klason, HCl, and Kraft lignins) used in this study are known to be highly modified from the natural state (11, 22). In addition, small amounts of carbohydrates may be associated with the lignin preparations (11). Therefore, the presence or absence of degradation of these substrates does not confirm or preclude the ability of the microorganism to degrade lignin in its natural state. Milled wood lignins and dioxane lignins have been characterized as being more representative of natural lignin, and they are preferred substrates for use in biodegradation studies (11). More bacterial isolates were capable of growing on milled wood lignin than any of the other lignin preparations.

A more definite test of the ability of an organism to degrade natural lignin is the use of specifically labeled [^{14}C]lignin-labeled lignocelluloses in biodegradation studies (11). The bacterium KB-1 demonstrated the ability to degrade the lignin component of the cordgrass *S. alterniflora*. This bacterium was capable of degrading four lignin preparations from peanut hulls, Kraft lignin from *P. elliottii*, and natural lignin of *Spartina* lignocellulose. The wide array of lignin substrates degradable by KB-1 indicates the versatility of the ligninolytic capability of this bacterium.

ACKNOWLEDGMENT

This research was supported by the Southeastern Peanut Association, Albany, Ga.

LITERATURE CITED

1. **Albrecht, W. J.** 1979. Peanut hulls: their properties and potential uses. U.S. Department of Agriculture agricultural research bulletin ARM-S-1. U.S. Department of Agriculture, Washington, D.C.
2. **Browning, B. L.** 1967. Methods of wood chemistry, vol. 2. Interscience Publishers, Inc., New York.
3. **Buchanan, R. E., and N. E. Gibbons (ed.).** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
4. **Cartwright, N. J., and K. S. Holdom.** 1973. Enzymic lignin, its release and utilization by bacteria. Microbios **8**:7–14.
5. **Chang, H., and K. V. Sarkanen.** 1973. Species variation of lignin: effect of species on the rate of Kraft delignification. TAPPI **56**:132–134.
6. **Crawford, D. L.** 1978. Lignocellulose decomposition by selected *Streptomyces* strains. Appl. Environ. Microbiol. **35**:1041–1045.
7. **Crawford, D. L., M. J. Barder, A. L. Pometto III, and R. L. Crawford.** 1982. Chemistry of softwood lignin degradation by *Streptomyces viridosporus*. Arch. Microbiol. **131**:140–145.
8. **Crawford, D. L., R. L. Crawford, and A. L. Pometto III,** 1977. Preparation of specifically labeled ^{14}C-(lignin)- and ^{14}C-(cellulose)-lignocelluloses and their decomposition by the microflora of soil. Appl. Environ. Microbiol. **33**:1247–1251.
9. **Crawford, D. L., S. Floyd, A. L. Pometto III, and R. L. Crawford.** 1977. Degradation of natural and Kraft lignins by the microflora of soil and water. Can. J. Microbiol. **23**:434–440.
10. **Crawford, D. L., and J. B. Sutherland.** 1979. The role of actinomycetes in the decomposition of lignocellulose. Dev. Ind. Microbiol. **20**:143–151.
11. **Crawford, R. L.** 1981. Lignin biodegradation and transformation. Wiley Interscience, New York.
12. **Gradziel, K., K. Haider, J. Kochmanska, E. Malarczyk,**

and J. Trojanowski. 1978. Bacterial decomposition of synthetic ^{14}C-labeled lignin and lignin monomer derivatives. Acta Microbiol. Pol. **27**:103–109.

13. **Haider, K., J. Trojanowski, and V. Sundman.** 1978. Screening for lignin degrading bacteria by means of ^{14}C-labelled lignins. Arch. Microbiol. **119**:103–106.
14. **Hodson, R. E., R. Benner, and A. E. Maccubbin.** 1983. Transformations and fate of lignocellulosic detritus in marine environments, p. 185–195. *In* T. A. Oxley and S. Barry (ed.), Biodeterioration 5. John Wiley & Sons, Inc., New York.
15. **Kerr, T. J.** 1979. Applications in general microbiology. Hunter Publishing Co., Winston-Salem, N.C.
16. **Maccubbin, A. E., R. Benner, and R. E. Hodson.** 1983. Interactions between pulp mill effluents and microbial populations in coastal waters and sediments, p. 246–256. *In* T. A. Oxley and S. Barry (ed.), Biodeterioration 5. John Wiley & Sons, Inc., New York.
17. **Maccubbin, A. E., and R. E. Hodson.** 1980. Mineralization of detrital lignocelluloses by salt marsh sediment microflora. Appl. Environ. Microbiol. **40**:735–740.
18. **Odier, E., G. Janin, and B. Monties.** 1981. Poplar lignin decomposition by gram-negative aerobic bacteria. Appl. Environ. Microbiol. **41**:337–341.
19. **Phelan, M. B., D. L. Crawford, and A. L. Pometto III.** 1979. Isolation of lignocellulose-decomposing actinomycetes and degradation of specifically ^{14}C-labeled lignocellulose by six selected *Streptomyces* strains. Can. J. Microbiol. **25**:1270–1276.
20. **Radhakrishnamurthy, B., and V. R. Srinivasan.** 1957. Studies on groundnut shells. I. Proximate analysis and the sugar make-up of the hemicellulose fractions. J. Sci. Ind. Res. (India) **16C**:59–61.
21. **Robinson, L. E., and R. L. Crawford.** 1978. Degradation of ^{14}C-labeled lignins by *Bacillus megaterium*. FEMS Microbiol. Lett. **4**:301–302.
22. **Sarkanen, K. V., and C. H. Ludwig.** 1971. Lignins: occurrence, formation, structure, and reactions. Wiley Interscience, New York.
23. **Sutherland, J. B., R. A. Blanchette, D. L. Crawford, and A. L. Pometto III.** 1979. Breakdown of Douglas-fir phloem by a lignocellulose-degrading *Streptomyces*. Curr. Microbiol. **2**:123–126.
24. **Trojanowski, J., K. Haider, and V. Sundman.** 1977. Decomposition of ^{14}C-labelled lignin and phenols by a *Nocardia* sp. Arch. Microbiol. **114**:149–153.