Clostridium sp. Associated with Discolored Tissues in Living Oaks

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ABSTRACT

Droplets oozing from discolored tissues on the end of bolts cut immediately after oak trees were felled contained an anaerobe, *Clostridium* sp., in addition to facultative bacteria. Phytopathology 61: 122-123.

Additional key words: discoloration and decay in living trees.

Although fungi have received most of the attention in the past, recent studies suggest that bacteria may play an important role in the processes that result in discoloration and decay in living trees (5, 6, 7, 8). Little is known about the species involved and their concn in tissues. We found that extremely high concn of bacteria do exist in certain discolored tissues in living trees; an anaerobe, *Clostridium* sp., is among them.

In 1967, drops of exudate were observed oozing from discolored xylem on the ends of recently cut bolts of red oak, *Quercus rubra* L., and chestnut oak, *Q. prinus* L. (Fig. 1, above), from the Cockoponset State Forest, Haddam, Conn. In 1969, similar droplets were observed again on the ends of recently cut bolts of red oak and white oak, *Q. alba* L., from the Massabesic Experimental Forest, Alfred, Maine. Approximately 20 trees of poor vigor having trunk wounds, poorly healed branch stubs, and obvious insect injuries in the trunks were cut and examined. The trees ranged from 10 to 30 cm in diam at 1.4 m aboveground. Droplets, white to yellow or sometimes pink, were visible on the cut ends of bolts from all trees approx 15 min after they were felled. Some oozed to 1.5 mm high. Most of the droplets were from discolored tissues associated with galleries made by wood-boring insects. The droplets formed most commonly on discolored tissues bordering tissues that were not discolored.

In the laboratory, discs were cut from 1-m bolts from several symptomatic trees cut 2 hr previously. A sterile gouge was used to extract small wood chips with the droplets on the surface. These chips were weighed and placed in sterile phosphate buffer and diluted serially. Spread plates were made on trypticase soy agar (TSA) and incubated 72 hr at 30 C under aerobic and anaerobic conditions. Anaerobic conditions were produced by the Weiss & Spaulding method (9) and by disposable $H_2 + CO_2$ generators. Two samples incubated under aerobic conditions contained 3.9×10^7 and 2.9×10^8 bacteria/g of wood. Under anaerobic conditions, the two samples contained 5.7×10^5 and 2.3×10^7 bacteria/g of wood. Most of these were facultative types.

Small chips of wood containing the droplets were transferred aseptically to fluid thioglycollate and incubated at 30 C for 5 days. Cultures were then streaked on TSA and incubated anaerobically for 4 days at 30 C. Cultures were transferred to fluid thioglycollate, streaked on TSA, and incubated under aerobic, anaerobic, and microaerophilic conditions. Several species of bacteria and yeasts grew from the droplets, but only one bacterium was an obligate anaerobe. It was gram-negative, motile, and formed spores (Fig. 1, below). The vegetative cells were approx 3.0×0.5 μ, and occurred mostly as single cells. The spores were terminal, resulting in a distention of the sporangium. Punctiform transparent colonies, approx 0.5 mm in diam, formed on TSA plates. Growth was enhanced on Bacto-AC medium where the colonies were approx 1 mm in diam. The bacterium was characterized according to tests outlined in the Manual of Methods for Pure Culture Study (2). The isolate weakly fermented D(+) levu-

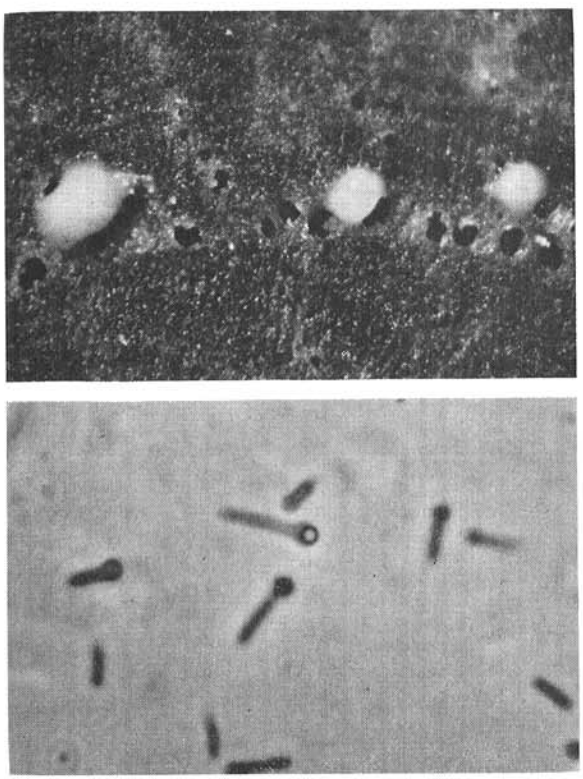

Fig. 1. (Above) Three white droplets of bacteria that oozed from discolored tissues in a red oak approximately 15 minutes after the tree was cut. The droplets are mucous-like (×30). (Below) The *Clostridium* sp. isolated from the droplets is shown here with its terminal spores (×5,000).

lose, inositol, and glycerol. Ribose was weakly fermented in the trypticase basal salts medium, but was not fermented in a veal infusion basal medium. Growth was generally better in the veal medium.

The bacterium did not produce hydrogen sulfide, acetoin, or indole; liquefy gelatin; lyse red blood cells; or reduce nitrate. The ability to hydrolyze cellulose was checked by a method modified after Maki (3). The culture did not hydrolyze starch or cellulose. The cultures were observed on egg yolk agar as described by McClung & Toabe (4). There was no change in the egg yolk medium, in litmus, or in whole iron milk media. There was no catalase or oxidase activity. The bacterium was placed in the genus *Clostridium,* as described in Bergey's Manual (1).

No bacteria were isolated from the healthy tissues surrounding the discolored tissues.

An independent study (by R. M. Smibert, Anaerobe Laboratory, and R. D. Brown, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg) with oak sections from the Massabesic Experimental Forest also indicated that a species of *Clostridium* was present. They stated that, under some nutritional conditions, anaerobic isolates from the diseased woody tissue gave rise to methane as did the tissue itself (R. M. Smibert, *personal communication*).

LITERATURE CITED

1. Breed, R. S., E. G. D. Murray, & N. R. Smith. 1957. Bergey's manual of determinative bacteriology. Williams & Wilkins Co., Baltimore, Maryland [7th ed.]. 1094 p.
2. Committee on Bacteriological Technique of the Society of American Bacteriologists. 1947. Manual of methods for pure culture study of bacteria. Biotech. Pub., Geneva, N. Y.
3. Maki, L. R. 1954. Experiments on microbiology of cellulose decomposition in a municipal sewage plant. J. Microbiol. Serol. 20:185-200.
4. McClung, L. S., & R. Toabe. 1947. The egg yolk plate reaction for the presumptive diagnosis of Clostridium sporogenes and certain species of the gangrene and botulism types. J. Bacteriol. 53:139-147.
5. McCreary, Mathilda, B. J. Cosenza, & A. L. Shigo. 1965. Bacteria isolated from decay and discoloration in northern hardwoods. Phytopathology 55:129-130 (Abstr.).
6. Shigo, A. L., & E. M. Sharon. 1968. Discoloration and decay in hardwoods following inoculations with Hymenomycetes. Phytopathology 58:1493-1498.
7. Shigo, A. L., & E. M. Sharon. 1970. Mapping columns of discolored and decayed tissues in sugar maple, Acer saccharum. Phytopathology 60:232-237.
8. Ward, J. C., J. E. Kuntz, & E. M. McCoy. 1969. Bacteria associated with "shake" in broadleaf trees. Phytopathology 59:1056 (Abstr.).
9. Weiss, J. E., & E. H. Spaulding. 1937. A simple method of obtaining effective anaerobiosis. J. Lab. Clin. Med. 22:726-728.