Discoloration and Fluorescence in Cotton Fiber Caused by Pseudomonas sp.

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ABSTRACT

A species of *Pseudomonas*, isolated from cotton bolls in Georgia, caused a green to yellow fluorescent discoloration in cotton fiber. Injury was necessary for entry of the bacterium into bolls. Optimum temperature for in vitro growth of the bacterium and pigment production in fiber was 30 C. The pigment was soluble in water, and its intensity of fluorescence decreased with field exposure.

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Various bacteria are commonly associated with developing and mature cotton bolls, and the literature relating them to boll rots was recently reviewed by Hunter (6) and Ashworth et al. (1). Little has been reported on the role of bacteria in reducing quality of fiber, although the fungi have received considerable attention (10, 14). A bacterium that causes a yellow to green fluorescent discoloration of fiber without causing significant boll rot is isolated commonly from bolls in Georgia. A description of this organism is presented here. An abstract on a portion of this work has been published (11).

In screening some 300 bacterial isolates collected in a boll rot survey made in Georgia during 1968-69 (12), we found 14 isolates from five different locations that did not cause either internal or external rot of bolls after puncture inoculation, but caused a discoloration in the fiber that fluoresced yellow to green under ultraviolet light (366 nm). This was of interest, as no other bacterium had been reported to cause fluorescent spots in cotton fiber. *Aspergillus flavus* is usually associated with similar spots (9). Additional isolations made from bolls collected in Georgia during 1970 and 1971 showed that the bacterium was associated with fluorescence in field bolls. Rot caused by other organisms sometimes masked the discoloration and reduced the intensity of fluorescence caused by the bacterium.

Entry of the bacterium into bolls and factors

influencing pigment production in fiber were studied in laboratory and greenhouse inoculations. A bacterial suspension (10^8 cells/ml) prepared from nutrient yeast dextrose agar (NYDA, Difco nutrient agar 23 g, yeast extract 5 g, and dextrose 10 g/liter distilled water) slants was atomized onto intact and excised mature (35 days old) green bolls, and excised natural or autoclaved (20 min at 15 psi) locks placed in sterile humidity chambers. Puncture inoculations (a blunt dissecting needle passed through a drop of suspension into boll) were made on intact and excised green bolls. Excised bolls were placed in sterile humidity chambers and supported upright with a wire grid, with their peduncles submerged in water. The bacterium produced a bright fluorescent-green to yellow discoloration in punctured intact or excised bolls and in atomized natural or autoclaved locks. No discoloration or other evidence of bacterial growth was found in atomized intact or excised bolls, indicating that a wound is necessary for bacterial entry. Puncture inoculations made on bolls 15 to 35 days old revealed that the bacterium would grow and produce fluorescence in bolls of all ages.

The influence of temperature on the in vitro growth of the bacterium and on fluorescence production in fiber was studied. Slants of NYDA streaked with the bacterium were placed in incubators at 5 to 40 C at 5-C intervals and observed after 24 hr. The bacterium grew best at 30 C. Slight growth occurred at 10 but not at 40 C. Petri dishes, each containing six aseptically excised locks atomized with the bacterium, were also placed at the eight temperatures. After 24 hr, the fluorescent pigment produced in the fiber was extracted with 100 ml of distilled water by soaking the locks for 1 hr. The fluorescence of the extract was measured with an Aminco Fluoromicrophotometer (American Instrument Co., Silver Spring, Md.) equipped with a 365-nm primary filter (Corning 7-37) and a 415-nm secondary filter (Wratten 47B). The instrument was calibrated so that the extract with the highest intensity gave a reading of 100 and other readings were relative to that value. Optimum temperature for pigment production was 30 C (Fig. 1).

The persistence of fluorescence in fiber after field exposure was also studied. Locks were removed aseptically from mature, unopened green bolls, placed in sterile plastic humidity chambers, and atomized with the bacterium, and the covered chambers were placed at 30 C. After 24 hr, the discolored locks were dried with a hair dryer, fluffed by hand, and placed in direct sunlight in petri dish lids (6 locks/lid). After 24-, 48-, 72-, and 96-hr exposure, the fluorescent pigment was extracted and measured fluormetrically as described above. Intensity of fluorescence decreased with exposure to sunlight, although the pigment was still visible and could be extracted after 96 hr of direct sunlight (Fig. 2).

In other tests, discolored and normal (controls) locks were attached to empty burrs on cotton plants in the field. The locks were examined at weekly intervals for 3 weeks during which ca. 2 cm of rainfall occurred. Field exposure decreased the intensity of

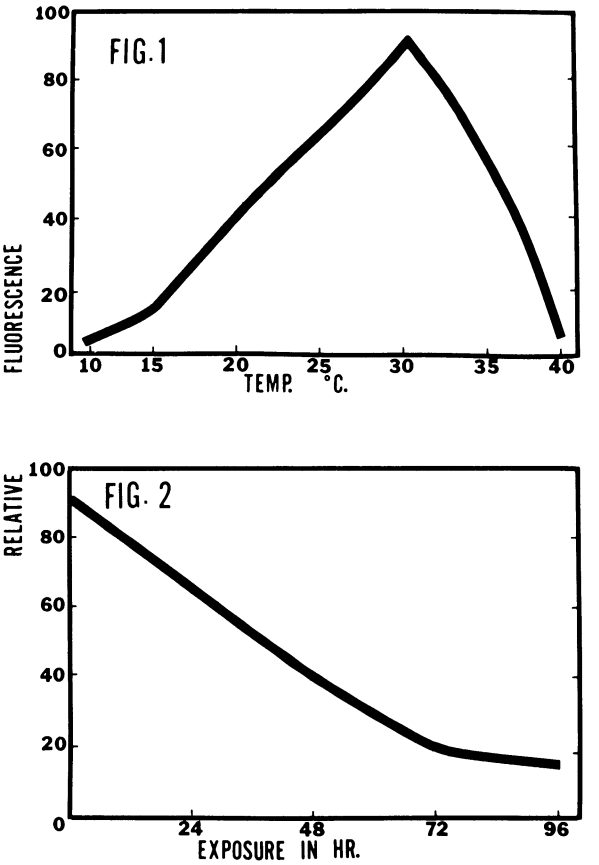

Fig. 1-2. Fluorescence in cotton fiber inoculated with a bacterium isolated from cotton bolls. Fluorescence intensity was determined by extraction of the pigment with water and measurement with an Aminco Fluoromicrophotometer. 1) Fluorescence in fiber inoculated and placed at different temperatures for 24 hr. 2) Fluorescent pigment remaining in locks after exposure to direct sunlight for various periods.

fluorescence. However, the fiber still fluoresced under ultraviolet light after 3 weeks' exposure. Decrease in intensity was greatest on the exterior part of the locks where the fiber became a dull green with little evidence of fluorescence. No fluorescence was observed in the control locks. These exposure studies indicated that the fluorescent pigment persists long enough to appear in the harvested fiber, although its intensity may be greatly diminished due to its photosensitivity and water solubility. Fluorescent fiber that undergoes long periods of field exposure appears as dingy green in the harvested product, a condition that is commonly attributed to the growth of various fungi.

Bacteria associated with cotton bolls have been studied mainly as causes of boll rot (1, 6). This bacterium and possibly others apparently cause a reduction in quality of fiber without causing direct losses through rot.

The bacterium was characterized in laboratory studies. Standard testing methods (3) were used

except where otherwise indicated. Nutrient agar colonies were circular to irregular, raised, smooth with undulate margins, and cream to buff in color. The bacterium was a gram-negative, nonsporeforming, motile (1-3 polar flagella) rod with a tendency to form a few chains of larger than normal cells. Physiological characteristics were as follows: aerobic; indole, hydrogen sulfide, and acetylmethylcarbinol not produced; gelatin not liquefied; starch, cellulose, and casein not hydrolyzed; rapid nitrite production from nitrate; catalase- and oxidase-positive (8); growth in 5% but not 10% NaCl broth; negative for methyl red, hypersensitive reaction on tobacco (7), 2-ketogluconate (13), potato soft rot test (8), lipase (13), arginine dihydrolase (13), β-glucosidase (4); milk changed to alkaline, no reduction, curd, or peptonization; citrate utilized as carbon source; acid but no gas from glucose, galactose, and xylose; oxidative but not fermentative use of glucose (5); no acid or gas from lactose, maltose, sucrose, rhamnose, or trehalose. A bright green to yellow fluorescent water-soluble pigment was produced on King B medium (15).

We have classified the organism as a member of the genus *Pseudomonas* (2). A specific name has not been assigned because of the unsettled nature of the taxonomy of this group (8, 13, 15). It apparently is a member of the saprophytic group of pseudomonads, and is probably of soil origin. The common association of the bacterium with bolls having insect injury suggests that insects may disseminate and introduce it into bolls.

LITERATURE CITED

1. ASHWORTH, L. J., JR., D. C. HILDEBRAND, & M. N. SCHROTH. 1970. Erwinia-induced internal necrosis of immature cotton bolls. Phytopathology 60:602-607.
2. BREED, R. S., E. G. D. MURRAY, & N. R. SMITH. 1957. Bergey's manual of determinative bacteriology [7th ed.]. Williams & Wilkins Co., Baltimore, Md. 1094 p.
3. COMMITTEE ON BACTERIOLOGICAL TECHNIQUE OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. 1957. Manual of microbiological methods. McGraw-Hill Book Co., New York. 315 p.
4. HILDEBRAND, D. C., & M. N. SCHROTH. 1964. β-glucosidase activity in phytopathogenic bacteria. Appl. Microbiol. 12:487-491.
5. HUGH, R., & E. LEIFSON. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-26.
6. HUNTER, R. E. 1968. A boll rot of cotton caused by Erwinia aroideae. Plant Dis. Reptr. 52:14-16.
7. KLEMENT, Z., G. L. FARKAS, & L. LOVREKOVICH. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
8. LELLIOTT, R. A., EVE BILLING, & A. C. HAYWARD. 1966. A determinative scheme for the fluorescent plant pathogenic pseudomonads. J. Appl. Bacteriol. 29:470-489.
9. MARSH, P. B., K. BOLLENBACHER, J. P. SAN ANTONIO, & G. V. MEROLA. 1955. Observations on certain fluorescent spots in raw cotton associated with the growth of microorganisms. Textile Res. J. 25:1007-1016.
10. MARSH, P. B., & M. E. SIMPSON. 1968. Occurrence of fiber containing fluorescent spots associated with Aspergillus flavus boll rot in the U.S. cotton crop of 1967. Plant Dis. Reptr. 52:671-674.
11. MC CARTER, S. M. 1971. Fluorescent spots in cotton fiber caused by a Pseudomonas species. Ga. Acad. Sci. Bull. 29:100 (Abstr.).
12. MC CARTER, S. M., R. W. RONCADORI, & J. L. CRAWFORD. 1970. Microorganisms associated with cotton boll rots in Georgia. Plant Dis. Reptr. 54:586-590.
13. MISAGHI, I., & R. G. GROGAN. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
14. SIMPSON, M. E., & P. B. MARSH. 1969. Microscopic observations on fungi associated with cotton boll-rot fibers. Mycologia 61:987-996.
15. STANIER, R. Y., N. J. PALLERONI, & M. DOUDOROFF. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43:159-271.