A Selective Medium for Isolating *Verticillium albo-atrum* from Soil

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ABSTRACT

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A defined, selective medium was developed for direct enumeration of *Verticillium albo-atrum* (dark mycelia type) propagules from soil and other substrates. The medium was modified from Komada's selective medium for *Fusarium oxysporum* and consisted of the following chemicals (per liter): L-sorbose, 2 g; L-asparagine, 2 g; K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Fe-Na-EDTA, 0.01 g; pentachloronitrobenzene (75% a.i.), 1 g; oxgall, 0.5 g; $NaB_4O_7 \cdot 10H_2O$, 1 g; streptomycin sulfate, 0.3 g; and the pH was adjusted to 5.7. Colonies of *V. albo-atrum* were more discrete and morphologically distinctive on this medium compared to other selective media. The recovery of propagules of *V. albo-atrum* from infested soil was significantly greater ($P = 0.05$) on this medium (68×10^3 propagules per gram of soil) than on a soil-extract agar or ethanol-streptomycin-penicillin agar (58×10^3 and 51×10^3 propagules per gram of soil, respectively).

Detection of *Verticillium albo-atrum* Reinke and Berth. (= dark mycelia type) propagules in soil is important in understanding the epidemiology of Verticillium wilt, particularly in a perennial crop like alfalfa. Development of selective media for *V. albo-atrum* has been largely ignored, and existing procedures (1,5) have not worked well under our conditions. A soil extract agar medium designed for *V. dahliae* was used successfully by Frank et al (4) to determine population densities of *V. albo-atrum* in the rhizosphere of potato; however, colonies of *V. albo-atrum* are difficult to recognize on this medium.

This paper describes a chemically defined selective medium on which colonies of *V. albo-atrum* can be readily identified.

MATERIALS AND METHODS

Komada's selective medium for *Fusarium oxysporum* (7) was modified as a selective medium for *V. albo-atrum*. L-sorbose, 20 or 2 g/L, or sodium polypectate (Grade II, Sigma Chemical Co., St. Louis, MO 63147), 2 g/L, were tested at C:N ratios of 10:1 or 1:1 as a substitute for D(+)galactose because they were used previously in media for *Verticillium* sp. Conidial suspensions of *V. albo-atrum* and *V. dahliae*, or of those species and *V. lateritium, V. nigrescens*, and *V. tricorpus* were pipetted separately onto the modified media. After 7 and 14 days, colony morphology was compared. The modified medium selected to enumerate soil populations of *V. albo-atrum* and to compare recovery with other selective media (2–4,6,8) consisted of K_2HPO_4, 1 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; Fe-Na-EDTA, 0.01 g; L-asparagine, 2 g; L-sorbose, 2 g; and distilled water, 1 L; to which pentachloronitrobenzene (75% a.i. [Terraclor 2 E.C., Olin-Mathieson Chemical Corp., Little Rock, AR 72203]), 1 g; oxgall, 0.5 g; $NaB_4O_7 \cdot 10H_2O$, 1 g; and streptomycin sulfate, 0.3 g; were added after the basal medium was sterilized. The pH of the liquid medium was adjusted to 5.3 by adding 4.5 ml of phosphoric acid (10%) to give a pH of 5.7 after solidification. The effect of media pH on recovery of *V. albo-atrum* was tested by varying the pH over a range of 4.6 to 8.4 with phosphoric acid or sodium hydroxide.

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Alfalfa field soil, naturally infested with *V. albo-atrum*, was collected at a depth of ~2–13 mm and processed immediately. One gram of soil, free from roots, was added to a 100-ml dilution bottle of 0.1% water agar and serially diluted to 1:1,000. One-milliliter aliquots of the dilutions were pipetted into five replicate petri dishes of a test medium and incubated at 20–25 C on a laboratory bench for 2 wk. Population density counts were analyzed as a randomized block design and mean separation was made according to Duncan's multiple range test, $P = 0.05$.

RESULTS AND DISCUSSION

Differences in colony characteristics after 7 days between *V. albo-atrum* and *V. dahliae* were greatest when these species were introduced on the media that contained 2 g L-sorbose at a 1:1 C:N ratio, or 2 g sodium polypectate at a 10:1 C:N ratio, but these differences were less distinct on the latter modification after 14 days. Colonies of *V. albo-atrum* on the sorbose medium were distinguished from *V. dahliae* by having twice the diameter, a hollow instead of compressed interior, and a smooth or lightly floccose instead of rough surface. Colonies of *V. albo-atrum* on this medium also were distinguishable from *V. nigrescens*, *V. lateritium*, and *V. tricorpus*. The colony characteristics of *V. albo-atrum* were alike at media pH 4.7, 5.7, and 6.1, but were variable at pH 6.6 or higher. Recovery of *V. albo-atrum* from a naturally infested soil was significantly greater ($P = 0.05$) at media pHs of 5.7 or 5.5 than at a pH of 4.6, with 21×10^3, 18×10^3, and 4×10^3 propagules per gram of soil, respectively.

Colonies of *V. albo-atrum* were recognized on the selective medium by shape, texture, and color. Cream-colored colonies from highly populated plates remained smooth or became floccose (Fig. 1A–C). The colonies were hollow and hemispherical until aerial mycelia spread down the side onto the agar surface. Colonies on sparsely populated plates became larger and developed brownish centers (Fig. 1D and E).

Propagules of *V. albo-atrum* were not detected on selective media described by Butterfield and DeVay (2), Jordan (6), or Zehsazian (8). A population count of *V. albo-atrum*, from naturally infested soil, on our selective medium was significantly greater ($P = 0.05$) than on soil extract agar or ethanol-streptomycin-penicillin agar, with 68×10^3, 58×10^3, and 51×10^3 propagules per gram of soil, respectively. Colonies on the soil-extract and ethanol-

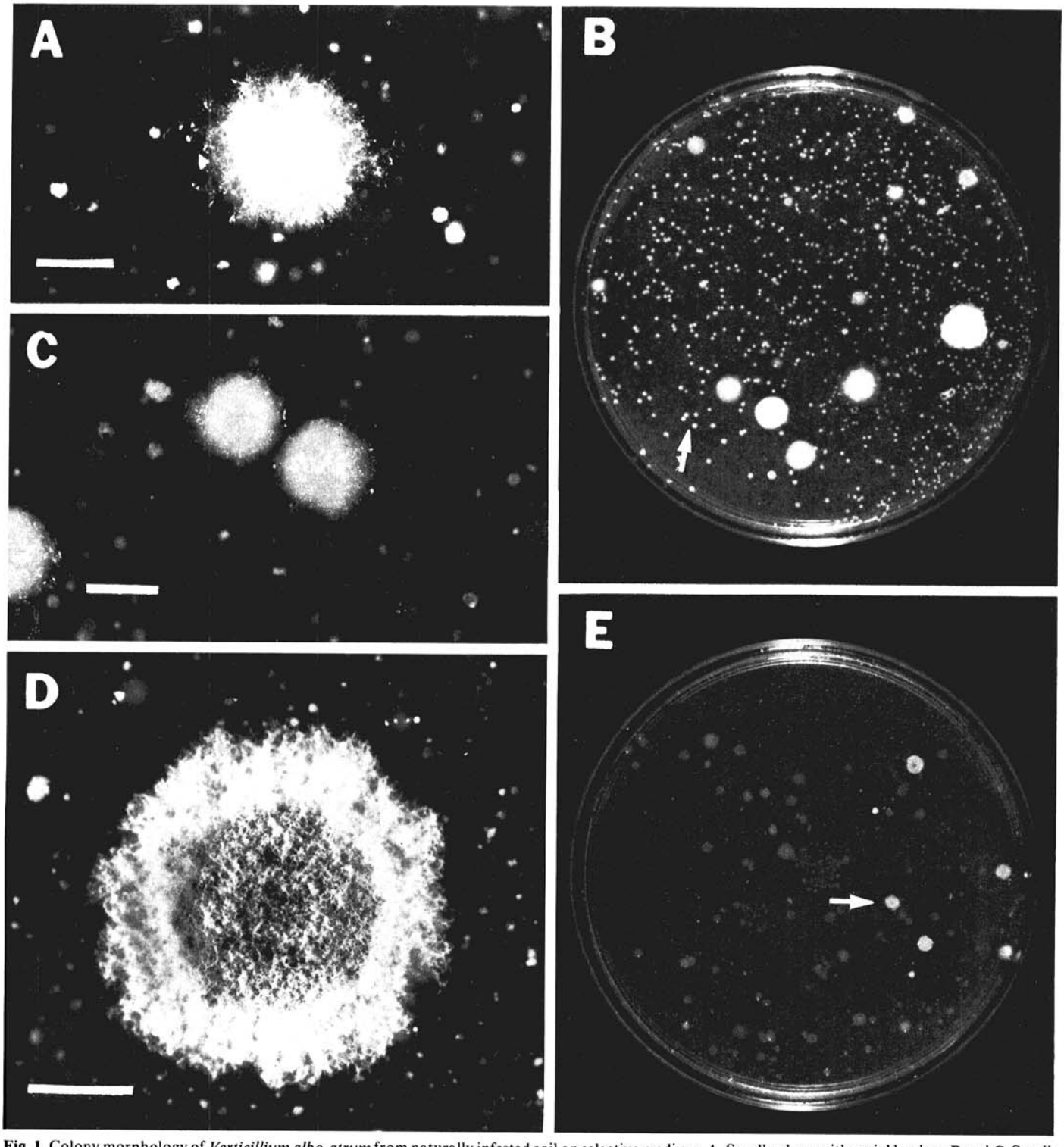

Fig. 1. Colony morphology of *Verticillium albo-atrum* from naturally infested soil on selective medium. **A,** Small colony with aerial hyphae. **B** and **C,** Small colonies with smooth surface on densely populated plates. **D** and **E,** Large colonies on low-populated plates. All scale bars = 1 mm.

streptomycin-penicillin agars were flat and translucent except for white centers. A few aerial conidiophores and dark hyphae were produced in the center of colonies on ethanol-streptomycin-penicillin agar. Nearly all colonies on our agar were discrete, whereas colonies on the soil-extract and ethanol-streptomycin-penicillin agars grew together and were difficult to count. Growth rate of *V. albo-atrum* on our medium was 0.6 mm/day at 25 C, whereas growth on the soil-extract agar and ethanol-streptomycin-penicillin agar was 3.7 and 3.3 mm/day, respectively. The selective agar described herein is an improved substrate for enumeration of propagules of *V. albo-atrum* from soil because colonies are easily discerned, and population counts are greater. We have successfully used the medium for isolation of *V. albo-atrum* from soil (1:500 and 1:1,000 dilutions), from the air, and from alfalfa seed.

LITERATURE CITED

1. Beckman, C. H. 1973. The incidence of *Verticillium* species in soils, vines and tubers of Rhode Island-grown potatoes. Plant Dis. Rep. 57:928-933.
2. Butterfield, E. J., and DeVay, J. E. 1977. Reassessment of soil assays for *Verticillium dahliae*. Phytopathology 67:1073-1078.
3. Easton, G. D., Nagle, M. E., and Bailey, D. L. 1969. A method of estimating *Verticillium albo-atrum* propagules in field soil and irrigation

waste water. Phytopathology 59:1171-1172.
4. Frank, J. A., Webb, R. E., and Wilson, D. R. 1975. The effect of inoculum levels on field evaluations of potatoes for Verticillium wilt resistance. Phytopathology 65:225-228.
5. Isaac, I., Fletcher, P., and Harrison, J. A. C. 1971. Quantitative isolation of *Verticillium* spp. from soil and moribund potato haulm. Ann. Appl. Biol. 67:177-183.
6. Jordan, V. W. L. 1971. Estimation of the distribution of *Verticillium* populations in infected strawberry plants and soil. Plant Pathol. 20:21-24.
7. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-124.
8. Zehsazian, H. 1966. An improved selective medium for the isolation of *Verticillium dahliae*. M.S. thesis. Oregon State University, Corvallis. 55 pp.