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Formation of pseudosclerotia and bacteria-induced chlamydospores in *Phoma foveata*

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Abstract

Isolates of *Phoma foveata* Foister from potato tubers produced pseudosclerotia and chlamydospores when grown on various agar media for two weeks or more. They also produced chlamydospores after two to seven days when in contact with four out of eighteen tested rhizosphere bacteria. Published keys and descriptions of *P. foveata* do not include the structures found.

In studies of varietal differences to infection of potato stems by the gangrene pathogen *Phoma foveata* (Kadir and Umaerus, 1987), and of spreading of this pathogen within the potato plant (Camyon, unpublished), formation of pseudosclerotia and chlamydospores were observed in some of the Petri dishes handled. Formation of these structures by *P. foveata* were not observed in studies on morphology, taxonomy and nomenclature of *Phoma exigua* Desm. and *P. foveata* Foister (Boerema and Höweler, 1967; Dorenbosch, 1970; Boerema, 1976; Boerema et al., 1987). The production of chlamydospores is an important diagnostic character of some species of *Phoma* (Dorenbosch, 1970) and also may affect survival ability and thus epidemiology, e.g. of *P. foveata*. We report here on experiments confirming the formation of pseudosclerotia containing chlamydospores in an agar medium, and of induction of chlamydospores in *P. foveata* by specific bacterial isolates from plant rhizospheres.

For observations of pseudosclerotia, six isolates of *P. foveata* from six different potato tubers (cv. Bintje), all harvested from different fields, were grown on agar in parallel tests. To ascertain pathogenicity on potato, the isolates were inoculated on potato tubers using the method of Kadir and Umaerus (1987). Identity was confirmed by observing production of anthraquinone pigment and yellow crystals on malt extract agar containing thiophanate-methyl (Topsin) (Tichelaar, 1974). One of the isolates was also identified by Centraalbureau voor Schimmelcultures (CBS) and is desposited under collection number CBS 557.97. Production of pseudosclerotia was examined for isolates grown at $20 \pm 2\,^{\circ}$C in the dark on potato dextrose agar (PDA), malt extract agar (MEA), potato-carrot agar (PCA), cornmeal agar (CMA), oatmeal agar (OMA) and water agar (WA). Mycelial fractions were taken from the agar plates at least twice a week for examination under the light microscope. The pseudosclerotia could also be observed through the bottom of inverted Petri dishes.

Dark brown to black pseudosclerotia mainly consisting of spherical to irregular chlamydospores were observed in all isolates (Figure 1). Brown, thick-walled terminal or intercalary chlamydospores were also produced in chains or in groups. The pseudosclerotia were very irregular in shape and 60 to 340 μm in diameter. They were observed on all media except on WA, but they were rare on OMA and sometimes not observed. No significant difference was observed between the six *P. foveata* isolates tested. On MEA, PDA, CMA and PCA the pseudosclerotia were usually found after 3–5 weeks of incubation. They, however, formed faster, after 2–3 weeks, when three or more colonies were grown on the same plate, and then mainly at

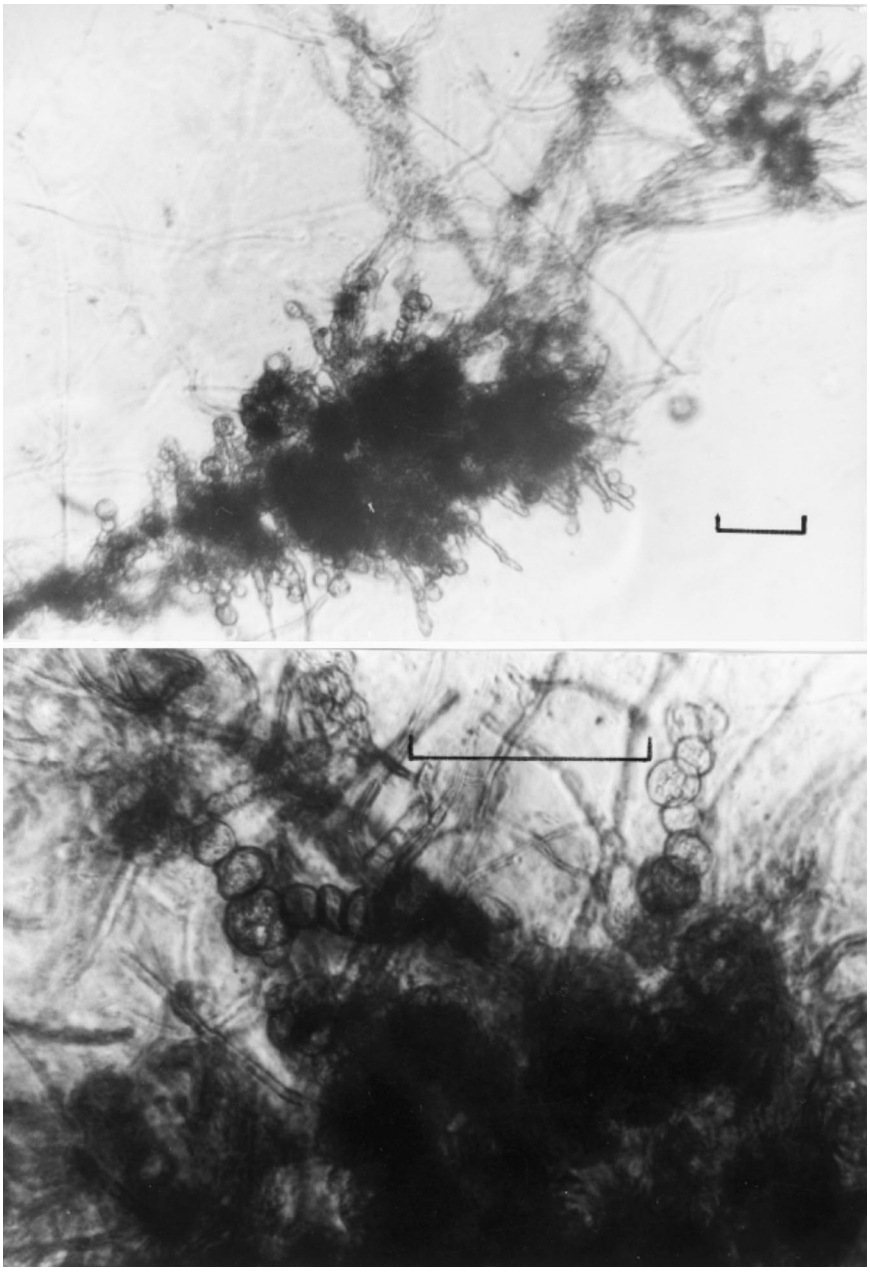

Figure 1. Brown to black pseudosclerotia, with chlamydospores from an agar culture of *Phoma foveata*. Bar on upper photo represents 400 μm and on lower photo 40 μm.

the periphery of the colonies. A general observation was that the older the culture the more pseudosclerotia could be observed.

For testing of bacterial induction, eighteen bacterial isolates from plant roots, identified by API 20 NE tests (API System Ltd., France) as *Serratia plymuthica* (6 isolates), *S. liquefaciens* (1 isolate), *Pseudomonas fluorescens* (7 isolates), *P. putida* (2 isolates) and as non-fluorescent *Pseudomonas* sp. (2 isolates), were used. They were isolated, stored and handled as earlier described (Gerhardson et al., 1985).

Effects of the bacteria on *P. foveata* were read at various intervals, after the margins of 3-day-old cultures of two of the *P. foveata* isolates, incubated at 15 °C in

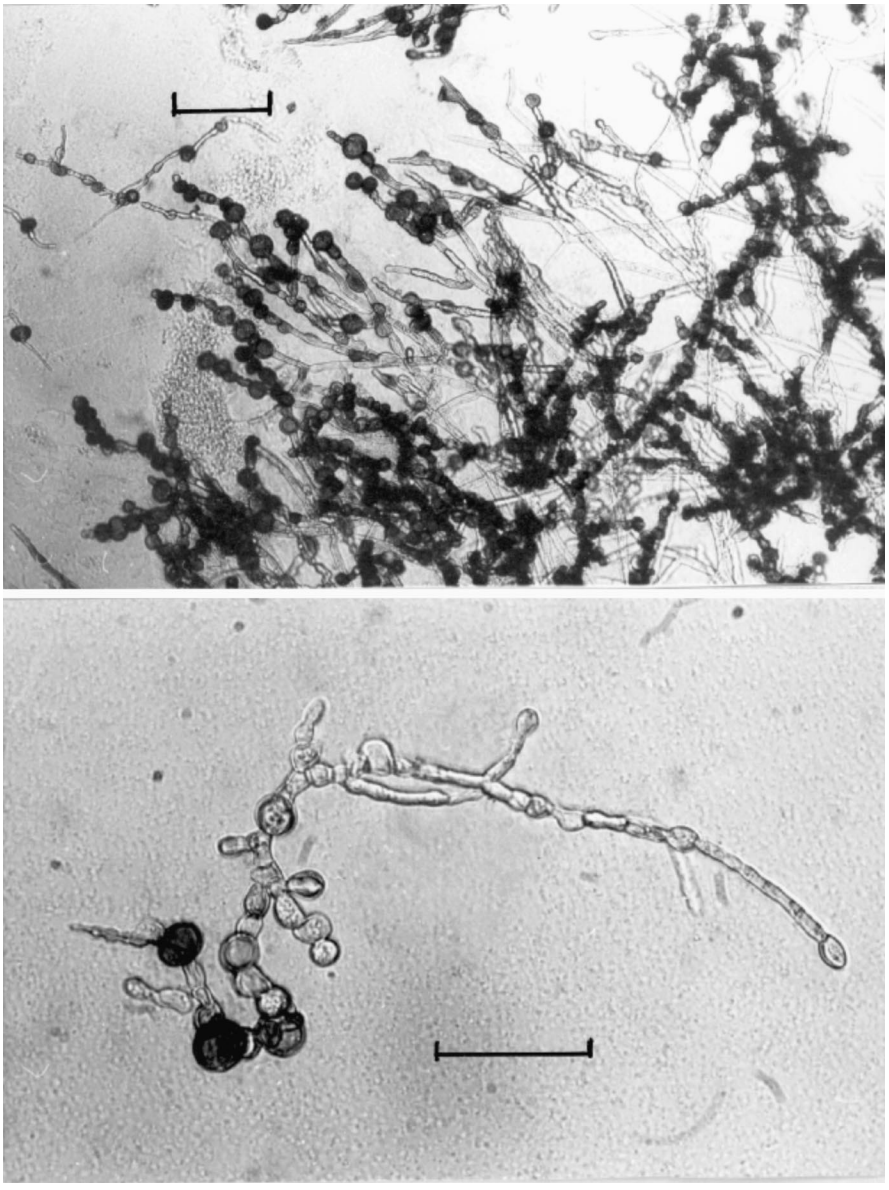

Figure 2. Bacteria-induced chlamydospores in chains from an agar culture of *Phoma foveata* coinoculated with a specific strain of the soil bacterium *Serratia plymuthica*. Bar on upper photo represents 100 μm and on lower photo 10 μm.

darkness, had been inoculated with a loop of a bacterial isolate. The bacterial inoculation material was taken from the surface of 2-day-old cultures grown on King's Medium B (Bergan, 1981). Tests were done on PDA, MEA, PCA and CMA. After inoculating the bacteria, the test plates were incubated at 15 °C in darkness and were then daily examined light-microscopically for 10 to 14 days. All experiments were repeated at least three times.

Four of the isolates of *S. plymuthica* regularly induced chlamydospore production in both isolates of *P. foveata*, 2 to 7 days after inoculation, especially one of the *S. plymuthica* strains was more effective than the others. The chlamydospores were dark brown to black with thick walls. They were spherical to irregular in shape, 1.8–3.7 μm in diameter, produced singly or in chains (Figure 2), and were initially not associated with pseudosclerotia. None of the other bacterial

species or the other two isolates of *S. plymuthica* tested induced chlamydospore production, which is evidence of a specific ability to induce chlamydospores.

A similar differential reaction of various fungal isolates to chlamydospore formation in mixed cultures with bacteria was reported by Ford, et al. (1970) in *Fusaritim solani* f. sp. *phaseoli.* Our findings support the hypothesis proposed by these authors that changes in numbers and types of bacteria may affect the ability to produce chlamydospores in a soil.

The gangrene pathogen *P. foveata* has been reported to survive in soil for up to five years in Northern Ireland (Khan and Logan, 1968; Anonymous, 1997) and for seven years in north-east Scotland (Malcolmson and Gray, 1968), but the survival structures in soil or on plants or plant debris are not well known. No sexual stage is known and the thin-walled conidia are less likely to survive for long periods. Probably thick-walled resting mycelium or chlamydospores, which can be seen in colonies on agar plates, are responsible for the survival (Adams, 1979), and the formation of pseudosclerotia described here add another probable survival structure.

Our observations of pseudosclerotia and chlamydospores give *P. foveata* characters adding to those given by Dorenbosch (1970) and Boerema and coworkers (1967, 1976, 1987). The pseudosclerotia described here in *P. foveata* are very similar to those produced by *P. chrysanthemicola* Hollós and described by Dorenbosch (1970).

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