Cytochromes in the Mycelium and Oospores of Phytophthora capsici

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ABSTRACT

Absorption spectra of the cytochromes in the vegetative and sexual propagules of *Phytophthora capsici* were obtained using a single-beam recording spectrophotometer, with vertical optics and on line with a computer. Cytochromes a, b, and c were detected in vegetative mycelium, whereas in young oospores (12-day-old cultures) or mature oospores (32-day-old cultures), only cytochrome c was detected.

Additional key words: oospore germination, respiration.

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Although many attempts have been made to increase germination of oospores of *Phytophthora* spp. (15), abundant germination is still elusive. Consequently, genetic studies of the genus have been seriously hampered (4, 11, 14). In much of the reported work, exogenous factors have been applied to stimulate germination of oospores, but despite some progress with this approach, little is known about the nature of dormancy.

Dormancy and germination of fungal spores have been related to characteristic changes in respiratory rates (12), but our preliminary studies did not detect respiration in dormant oospores. Since germination of oospores is low as well as nonsynchronous, meaningful studies of respiratory rates of germinating oospores were not feasible. We therefore, looked spectrophotometrically for cytochromes in the mycelium and in dormant oospores of *Phytophthora capsici* Leonian as indicators of the cellular respiratory chain.

MATERIALS AND METHODS.—Oospores of the heterothallic *P. capsici* (P504 A^1 × P505S A^2) (14), were produced in 90-mm diameter petri dishes which contained 15-20 ml V-8 juice (Campbell's Soup Co.) neutralized with calcium carbonate (5g $CaCO_3$/354 ml V-8 juice). The medium was clarified by centrifugation (14). Each dish was seeded with 1 ml of minced mycelium of each of the A^1 and A^2 mating types, and incubated in the dark for 12 or 32 days at 24 ± 1 C. Oospores were aseptically harvested by comminuting mycelial mats in a Waring Blendor and sieving through nylon mesh (53 μm pore size). The filtrate was centrifuged several times, at 1,000 *g* to separate mycelial fragments from the oospores.

Cytochromes in oospores and mycelium were measured with a single-beam recording spectrophotometer, consisting of a Cary 14 monochromator on line with a PDP-8/I computer. Technical details of the spectrophotometer have been described previously (2). The samples were frozen to 77 K with liquid nitrogen to intensify the absorption bands. Mycelium (31 μg protein/mg dry weight) or oospores (27 μg protein/mg dry weight) (10), were placed in the cuvette together with 0.5 g $CaCO_3$, which served as a light-scattering agent to increase the optical pathlength (1). A vertical light path was employed to ensure that the sample would remain in the measuring beam throughout the experiment.

RESULTS.—Absorption spectra typical of cytochromes a, b, and c were observed in the mycelium of *P. capsici* (Fig. 1). The a-type cytochromes exhibit a broad peak with absorption maxima between 592-597 nm. The b-type cytochromes have a broad peak at 557 nm, and the c-type cytochromes have broad peaks at 546 and 543 nm. Higher derivative analysis (Fig. 1, curve B) of the spectrum (Fig. 1, curve A), resolved the alpha and beta absorbance bands of the b- and c-type cytochromes. Since the amplitude of the fourth derivative band is inversely proportional to the fourth power of the band width of the original curve (3), the derivatizing intervals (1.6, 1.8, 2.0 and 2.4 nm), were chosen to resolve the relatively narrow alpha and beta absorbance bands of the b- and c-type cytochromes. The intervals discriminate against the broad absorbance band between 592-597 nm. The b-type cytochromes, resolved by derivative analysis of the low-temperature spectrum, showed absorption maxima at 557 and 564 nm, and the c-type cytochromes showed absorption maxima at 543, 552, and 556 nm. These observations are consistent with previous reports of cytochromes in oomycetes (5, 6).

Absorption spectra of cytochrome c were detected in both young oospores (12-day-old cultures), and mature oospores (32-day-old cultures) (Fig. 2). No other cytochromes were detected. Observations of spectra after fracturing the oospores by the method of Lippman et al (9), did not result in any alteration of the cytochrome pattern observed, thus indicating that penetration of the light beam through the dense oogonium and oospore walls was not a limiting factor in the analysis.

DISCUSSION.—Little is known about the presence and role of cytochromes in dormant fungal spores. Sussman and Douthit (13) suggest that there may be an involvement of the first part of the electron transport

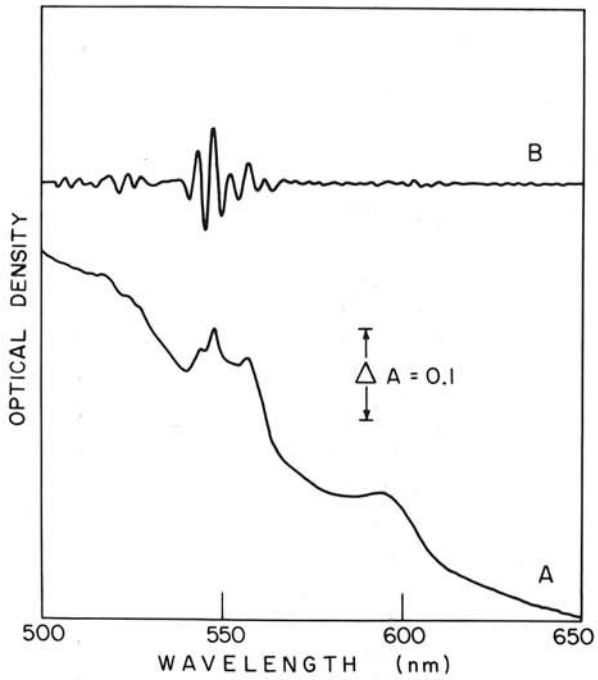

Fig. 1. Absorption spectrum of mycelium of *Phytophthora capsici* at 77K. Curve A, reduced with sodium dithionite. Curve B, computer derivative analysis of curve A. See text for explanation of curve B.

chain in the breaking of dormancy of bacterial spores. However, they point out that the presence of reduced cytochromes in bacterial spores and the failure to block germination by inhibition of cytochrome oxidase, seem to rule out any major role for that portion of the chain. In dormant fungal spores, much less is known of the possible role of the electron transport system in the germination process. If a complete electron transport chain is necessary to initiate and/or maintain the germination process, then our findings may partially explain the low germinability of *P. capsici* oospores. Incomplete development of the respiratory chain may also be one of the contributory factors for the problem encountered with *P. infestans* oospores that germinated, but subsequently failed to become established as colonies (4, 11). Recently, Hemmes and Bartnicki-Garcia (7) indicated that mitochondria disintegrate or decrease in number during oosporogenesis. Their electron micrographs of dormant oospores also suggest that much cell differentiation must occur during the germination process. Also, Leary et al. (8), have reported the lack of typical intact functional 80S ribosomes, a basic component of protein synthesis, in dormant oospores of *Phytophthora*. However, subribosomal ribonucleoprotein particles, ribosomal RNA, and ribosomal protein were isolated from oospores, suggesting that intact ribosomes may not be required for the initial germination process. Our results, coupled with those of Hemmes and Bartnicki-Garcia (7) and Leary et al. (8) may provide some insight into the problem of dormancy and germination of oospores of *Phytophthora* spp.

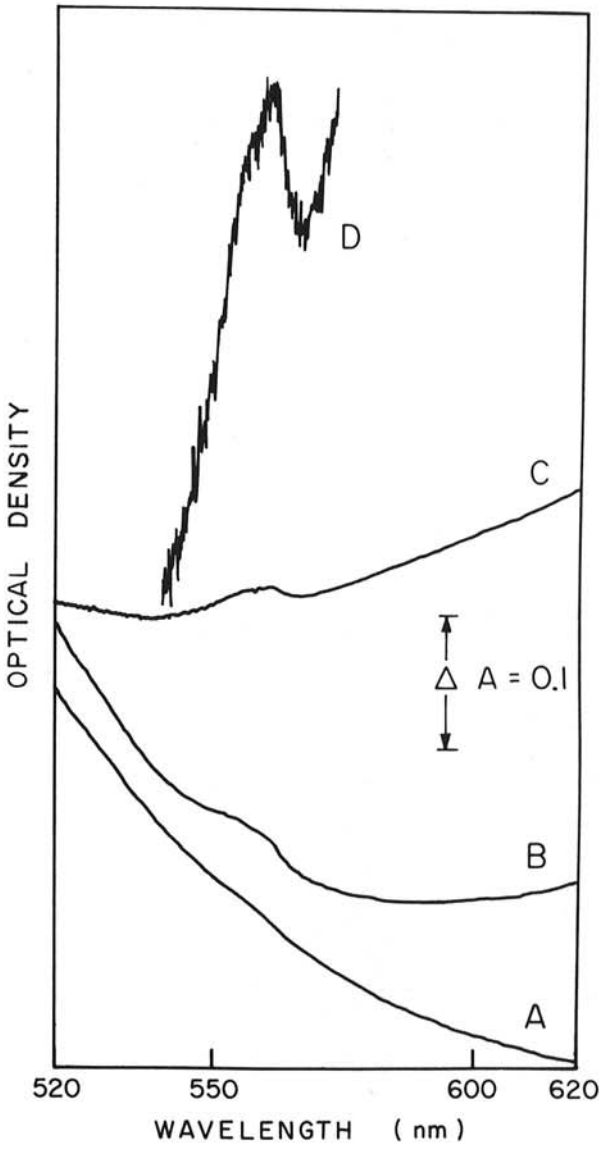

Fig. 2. Absorption spectrum of intact mature oospores of *Phytophthora capsici* at 77K. Curve A, oxidized spectrum. Curve B, same sample reduced with sodium dithionite. Curve C, computer-derived, reduced-oxidized spectrum. Curve D, derivative analysis of the spectrum.

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