The conidial surface of *Botrytis cinerea* and several other *Botrytis* species

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Abstract: Surfaces of conidia of *Botrytis cinerea* Pers. Fr. and several other *Botrytis* species were studied using scanning electron microscopy and transmission electron microscopy with carbon-platinum replicas. The surface of dry conidia of *B. cinerea* was rough with numerous short (200-250 nm) protuberances. Upon hydration and redrying these protuberances disappeared. The surfaces of conidia of other *Botrytis* species were similar to the surface of *B. cinerea*. The basket-weave pattern of hydrophobin roddets present on the surfaces of spores of many fungal species was not observed on conidia of any of the *Botrytis* species. Roddets were seen when the methods employed in this study were used to examine conidia of *Aspergillus nidulans* (Eidam) Winter, *Neurospora crassa* Shear and Dodge, or *Penicillium camembertii* Thom.. fungal species known to possess roddets.

Key words: hydrophobicity, conidial surface, *Botryotinia*, electron microscopy.


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Introduction

Conidia of the deuteromyecete *Botrytis cinerea* Pers. Fr., a serious fungal pest of many plants and plant products, are very hydrophobic (Doss et al. 1993). Indeed, it is likely that conidial adhesion of *B. cinerea* depends on hydrophobic interactions between the spore and the plant surface (Doss et al. 1993).

Conidia from at least three fungal classes (Ascomycetes, Deuteromycetes, and Basidiomycetes) can exhibit a basket-like pattern of roddets on their surface (Hawker and Madelin 1976). These roddets are composed of a class of proteins called hydrophobins that appear to be responsible for conferring cell surface hydrophobicity onto spores and other aerial fungal structures (Beever and Dempsey 1978; Stringer et al. 1991; Stringer and Timberlake 1993; Wösten et al. 1993).

Earlier work, in which freeze-etching was used to study the surface of conidia of *Botrytis fabae*, failed to reveal the arrays of roddets seen with spores of many other fungal species (Epton and Richmond 1980; Richmond and Pring 1971). Moreover, the conidial surface of *B. fabae*, unlike the surfaces of many fungal spores (Hawker and Madelin 1976), lacked ornamentation of any kind. Reported here are results of a study carried out to learn more about the conidial surface of *B. cinerea* and other *Botrytis* species. Carbon-platinum replicas of dry and hydrated (and redried) conidia were examined with a transmission electron microscope. The scanning electron microscope was used to examine gold-palladium-coated specimens. Conidia of four other *Botrytis* species were examined, as were conidia of three fungal species known to possess hydrophobin roddets (as a test of the methods).

Materials and methods

Conidia from an isolate of *Botrytis cinerea* (Be-1) used in several earlier studies (Doss et al. 1993, 1994) were obtained as described
previously. Petri plates containing potato dextrose agar (PDA) (Difco, Detroit, Mich.) were inoculated with a conidial suspension, and cultures were maintained at 20°C under near-UV light for 10–14 days. Conidia were collected on the lid of an inverted Petri plate culture by gentle tapping of the plate bottom. Conidia were hydrated by adding water to the Petri dish lid and mixing the suspension. Similar methods were used to obtain conidia from cultures of Botrytis fabae Sardiña (ATCC 28476).

Botrytis aclada Fresenius (isolate 61-2, provided by J.W. Lorbeer, Cornell University, Ithaca, N.Y.) and an isolate of Botrytis tulipae (Libert) Lind., which was originally isolated from cv. Preludium tulip leaves, were cultured using methods similar to those used for B. cinerea and B. fabae, except cultures were begun with mycelial plugs instead of conidial suspensions. Conidia of Botrytis elliptica (Berk.) Cook, isolate DG 3-7, were obtained by culturing the fungus on leaves of field-grown Asiatic lilies (cv. Antarctica) using methods described previously (Hsiang and Chaustagner 1991).

Because they were known to exhibit hydrophobic rodlets (Hallett and Beever 1981; Hess et al. 1968; Stringer et al. 1991), conidia of Aspergillus nidulans (Eidam) Winter (ATCC 10074), Neurospora crassa Shear et Dodge (ATCC 14692), and Penicillium camemberti (ATCC 4845) were also examined. Aspergillus nidulans and P. camemberti were cultured on PDA. Neurospora crassa was cultured on Neurospora Minimal Medium (Jong and Edwards 1991).

The method described by Boucias et al. (1988) was used to prepare carbon—platinum replicas for transmission electron microscopy. Dry conidia were dusted onto the split mica substratum used for coating; hydrated conidia were taken up in water, immediately applied to the mica as an aqueous suspension, and dried prior to coating. A Philips (CM12-STEM) transmission electron microscope operated at 60 keV was used to examine replicas.

Scanning electron microscopy was carried out after coating either dry, unfixed conidia with 60% gold – 40% palladium, or after similar coating of conidia deposited as an aqueous spore suspension. Examination was made with an Amray 1000A scanning electron microscope operated at 30 keV. A JEOL field emission microscope operated at 2.3 keV was also used to examine unhydrated conidia of B. cinerea.

**Results and discussion**

Surface replicas made using conidia of Aspergillus nidulans, Neurospora crassa, or Penicillium camemberti exhibited rodlet patterns similar to those described previously (Figs. 1–3) (Hallett and Beever 1981; Hess et al. 1968; Stringer et al. 1991). This was true with replicas prepared from either unhydrated conidia, or conidia deposited as an aqueous spore suspension. Freeze-etching has usually, but not always, been used to observe hydrophobic rodlets. Results obtained in this study demonstrate that carbon—platinum replicas of hydrophobic rodlets can also be readily prepared, and that hydration (as required for the freeze-etching process) does not influence their appearance.
Fig. 4. Images produced from replicas of unhydrated conidium of *Botrytis cinerea*. Processes are indicated by arrows. Scale bar = 1 μm. Inset shows replica of conidial margin at a higher magnification. Scale bar = 100 nm. Fig. 5. Field emission scanning electron micrograph of unhydrated conidia of *B. cinerea*. Processes indicated by arrows. Scale bar = 1 μm. Inset shows higher magnification. Scale bar = 100 nm.

With replicas prepared using hydrated conidia of *N. crassa*, the pattern of hydrophobin rodlets extended beyond the images of the spore margin (Fig. 1). This was not true when replicas were prepared from dry spores. Wösten et al. (1993) recently demonstrated that self-assembly of a hydrophobin protein from *Schizophyllum commune* can occur, to give the typical rodlet
pattern. They speculated that hydrophobin also self-assembles on aerial hyphae and inert substrata. It is possible that the rodlet pattern observed beyond the spore margin with *N. crassa* replicas arose from hydrophobin that was dislodged from the conidia during preparation of the spore suspension and that reassembled upon the split mica used for replica preparation (Fig. 1). (Replicas of conidial margins were not obtained with the other rodlet-forming fungi.)

Replicas of the surface of dry conidia of *Botrytis cinerea* did not exhibit a rodlet pattern. Rather, they revealed a surface roughened by the presence of numerous cylindrical protuberances (about 40 nm in diameter by 200 nm long) (Fig. 4). Field emission scanning electron microscopy of intact conidia suggested a similar structure (Fig. 5). Unhydrated conidia examined using a standard scanning electron microscope had a similar appearance. This is the first report of the presence of protuberances on conidia of *Botrytis* species. All earlier images of the conidial surface of *Botrytis* species were obtained with conidia that had been hydrated.

Hydration caused a change in conidial appearance, eliminating the protuberances and producing a surface characterized by irregular pits and bumps (Fig. 6). At high magnification, replicas prepared from hydrated conidia exhibited a graininess suggesting a coating of particles (Fig. 6, inset). Surface replicas of the conidial surface of *B. cinerea* closely resembled the freeze-etched surfaces of conidia of *B. fabae* described by Richmond and Pring (1971). Again, scanning electron microscopy of hydrated conidia revealed a surface structure similar to that inferred from the carbon–platinum replicas (Fig. 7).

Micrographs of replicas prepared from other *Botrytis* species are shown in Figs. 8–11. These images, selected for presentation on the basis of overall quality, show the cylindrical protuberances less clearly than does the replica of *B. cinerea* illustrated in Fig. 4. Nevertheless, the surfaces inferred from the replicas are very similar, and protuberances are present. In no case were replicas of rodlets seen, and with each species the conidial surface was markedly changed by hydration (i.e., protuberances present on dry conidia, but absent from hydrated conidia).

Evidence suggests that the fungal hydrophobins, a widely distributed class of hydrophobic proteins that differ markedly from one another in amino acid sequence but possess several common characteristics (Stringer and Timberlake 1993), form the rodlet layer on conidia of several fungal species (Stringer and Timberlake 1993). Their presence appears to confer the hydrophobicity exhibited by the conidia (Beever and Dempsey 1978; Chasan 1991; Stringer et al. 1991). Hence, given the hydrophobic nature of conidia of *B. cinerea* (Doss et al. 1993), and the fact that the conidial surface of *B. fabae* is proteinaceous (Fisher and Richmond 1969), it would not have been surprising to observe the typical rodlet pattern with conidia of *Botrytis* species. The absence of such a pattern on conidia of *Botrytis* species, and on spores from a number of other fungi, means that spore surface hydrophobicity can be conferred by materials other than the rodlet-forming hydro-
Figs. 8–11. Images produced from carbon–platinum replicas of dry and hydrated conidia of four Botrytis species. Fig. 8. Botrytis aclada. Fig. 9. Botrytis elliptica. Fig. 10. Botrytis fabae. Fig. 11. Botrytis tulipae. In each case the inset shows an image derived from the hydrated surface. Scale bars = 100 nm. Arrows in insets in Figs. 10 and 11 indicate replicas of conidial margins.

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References


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