SHORT COMMUNICATIONS

Surface Ultrastructure of Oidia in the Basidiomycete *Psathyrella coprophila*

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The existence of oidia has long been known and their structure and development have been described using the light microscope (Brodie, 1936). They are formed asexually, are uninucleate and usually borne in clusters on the monokaryotic mycelia of many basidiomycetes. Oidia of different species may have different functions. In some species they readily germinate to form monokaryotic mycelia. In many coprophilous species they do not germinate (Falck, 1902), and they probably act as fertilizing agents or spermatia. The oidia of *Psathyrella coprophila* Watling (Watling & Jurand, 1971), which were used in this investigation, do not germinate. They are borne on oidiophores and cohere in a droplet of mucilage.

The surface structure of oidia is of interest in studies of the relationship between the hyphae and the oidia. Hyphae of many species are known to react to an oidial stimulus by growing towards and fusing with oidia (Bistis, 1970; Kemp, 1970). The directed growth and fusion between hyphae and oidia is either followed by dikaryotization or by vacuolation and death. Dikaryotization indicates that two isolates belong to the same species. Homing followed by a lethal reaction indicates that two isolates are closely related. The physiological basis for the recognition of oidia by hyphae has not yet been resolved and a study of the oidial surface might be relevant to this problem.

From this preliminary study it is suggested that the surface of the oidia of *Psathyrella coprophila* has numerous filamentous appendages which may be embedded in a layer of mucilage. A similar surface structure has been described in certain bacteria. In some respects the filamentous appendages appear to resemble bacterial fimbriae (pili) (Duguid, Smith, Dempster & Edmunds, 1955; Brinton, 1965), and the mucilaginous coat in which they are embedded may be similar to bacterial capsules (Duguid, 1951).

METHODS

Psathyrella coprophila was chosen because it produces abundant oidia in culture. Monokaryotic mycelia, from basidiospores of the type specimen which is deposited in the herbarium of the Royal Botanic Garden, Edinburgh, were grown on agar plates at 20 °C on horse-dung extract agar (Lange, 1952). The oidia were harvested when the mycelium almost covered the agar surface.

For electron-microscope preparations the oidia were harvested with 0.81% (w/v) NaCl. About 4 ml of the saline solution was poured into each plate, the oidia suspended by gentle rocking and the suspension centrifuged at 3000 rev./min for 5 min to concentrate the oidia into a pellet. Three fixation methods were used:

(i) *Fixation with OsO₄* (1%, w/v) according to Palade (1952), but with addition of 0.045 mg sucrose/ml of fixative, for 1 h at room temperature;

(ii) *Double fixation with glutaraldehyde and OsO₄*. The oidia were fixed for 1 h at 0 to 2 °C with glutaraldehyde (2.5 %, v/v) in 0.1 M-phosphate buffer at pH 7.2, then washed with 0.1 M-phosphate buffer and fixed with OsO₄ (1 %, w/v) dissolved in the buffer;

(iii) *Double fixation in the presence of ruthenium red*. The fixation procedure used was that of Luft (1965). It has been described in detail by Pate & Ordal (1967).

After fixation by methods (ii) and (iii) the pellets were rinsed with buffer, dehydrated with a graded series of alcohol solutions, passed through 1,2-epoxy propane and embedded in Araldite. Fixation method (i) was followed, without rinsing, by dehydration and embedding. Sections were prepared with glass knives on an LKB Ultratome. Sections of the osmium-fixed material were stained with 2.5 % (w/v) uranyl acetate and 1 % (w/v) potassium permanganate for 20 min (Lawn, 1960). The sections of the double-fixed materials, (ii) and (iii), were stained with 1 % (w/v) uranyl acetate solution for 30 min and with lead citrate solution (Reynolds, 1963) for 15 min. The stained sections were examined with an AEI EM6B electron microscope.

For light-microscope observations the oidia were suspended in distilled water, concentrated by centrifugation and relief stained with indian ink (Duguid, 1951).

RESULTS

Sections of oidia prepared after fixation with OsO₄ and those prepared after double fixation showed filamentous appendages on the surface of the oidia (Fig. 1*a, b*). The filaments were similar in size and shape after both (i) and (ii) methods of fixation. Their diameter and length were approximately 10 nm and 0.5 µm respectively and they appeared to taper slightly. The structure of the appendages differed after the two fixation methods. After osmic fixation the filaments seemed to be hollow while those after double fixation appeared solid and more heavily stained. Also in the osmic-fixed material some of the filamentous appendages seemed to pass through the oidial wall and the plasmalemma but this was never seen in the double-fixed material.

Fig. 1*a, b* clearly indicate the presence of the filamentous appendages but give no indication of an extracellular capsule. In the indian ink preparations examined by the light microscope the capsule was seen as a light band surrounding the oidia (Fig. 1*c*). The capsule was similar to those found in many bacteria (Duguid, 1951). The ruthenium-red preparations also indicated the presence of a capsule (Fig. 1*d*). The region positive for ruthenium red that surrounded each oidium was similar in width to the light band which was seen in the light microscope preparations and to the length of the filaments seen by the fixation techniques (i) and (ii). A fibrillar component was also present in the ruthenium-red positive region but there were no distinct appendages. The capsule probably contained some highly polymerized acid mucopolysaccharides because the affinity of ruthenium red to these surface carbohydrates is well documented (Luft, 1964).

Of the internal structures of the oidia only the major organelles were clearly distinguishable. A single, large nucleus occupied almost half of the cell section. All the cell membranes and especially the nuclear envelope were most clearly shown after osmic fixation. The mitochondria were large in proportion to the size of the oidium, and were elongated and few in number. All the oidia contained at least one prominent inclusion which was also visible in the light microscope. These inclusions were probably lipid structures of the type referred to by Hawker (1965).

The final shape of the oidia indicates the method of their formation. Some of them had one flat and one rounded end (Fig. 1*a*), and others had two flat ends (Fig. 1*b*). The flat ends

Fig. 1 (a) Electron micrograph of a longitudinal section of an oidium double-fixed in glutaraldehyde osmic acid, showing filaments (Fl), and nucleus (N).

(b) Electron micrograph of a longitudinal section of an oidium fixed in osmic acid only, showing filaments (Fl) and nucleus (N).

(c) Photomicrograph of oidia relief stained in indian ink, showing capsule.

(d) Electron micrograph of a section of the capsule region of oidium double fixed with glutaraldehyde and osmic acid in the presence of ruthenium red.

had terminal flanges. Oidia of the first type were produced as the terminal sections of the finger-like outgrowth of the oidiophore, while the others were intercalary.

DISCUSSION

Several different techniques were used in order to reveal the surface structure of oidia but the relationship of the filamentous appendages to the capsule is still not clear. No single preparation has yet shown the filamentous appendages together with the capsule.

There are two possible explanations of the results presented in this communication. Either the filaments were true filamentous organelles present in the living organism, or they were artifacts produced during the fixation or dehydration of the capsule. There is some evidence which argues against the possibility of artifacts. Two different fixation techniques give comparable results (Fig. 1*a, b*), and although dehydration of the capsule could lead to the formation of filaments, it is doubtful that such regularity in form and thickness could be achieved by simple drying. There was some indication that in a few places filaments passed through the oidial wall and plasmalemma into the oidium, but the relevant micrographs lacked contrast and would not reproduce satisfactorily. Perhaps the failure to show a clear continuation of the filaments through the wall is due to their small diameter in relation to the thickness of the sections.

The observations of the surface structure presented here are by no means unique. Similar observations and difficulties in interpretation have been described in many bacteria. The term fimbriae which was originally used to describe rigid, hairlike appendages on the surface of many capsulate and non-capsulate bacteria (Duguid *et al.* 1955) has now been broadened to include branches and flexuous filaments (Yanagawa & Otsuki, 1970; Wistreich & Baker, 1971). Some of these strongly resemble the filamentous appendages described in this report.

Bacterial fimbriae are usually made of single proteins and often possess antigenic properties. The chemical nature of the oidial filamentous appendages is not yet known and further work is necessary before more certain interpretations of this work can be put forward.

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