The Morphology of a Corynebacterium sp. Parasitic on Annual Rye Grass

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ABSTRACT

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The *Corynebacterium* species, which parasitizes *Lolium rigidum*, is surrounded by a capsule approximately 0.2 μm thick that has specific adhesive properties enabling the bacterium to stick to the cuticle of its mechanical vector, the second-stage larva of the nematode *Anguina lolii*. The bacterium swells upon hydration. Exclusive of its capsule the anhydrobiotic organism is about two-thirds of the diameter of the hydrated organism. However, the overall size of the capsulated bacterium remains constant because the capsule is thinner in the hydrated form.

All Gram-positive phytopathogenic bacteria heretofore described belong to the genus *Corynebacterium*. The organism that we describe in this paper parasitizes annual rye grass (*Lolium rigidum* Gaudin, 1811) and some related species (5). It occurs as a yellow slime or in yellow galls which are thought to contain the toxic component in a fatal disease of sheep and cattle associated with summer grazing on rye grass (4).

Natural infection of rye grass with this *Corynebacterium* sp. does not occur in the absence of a nematode (*Anguina lolii*) which is thought to act as a vector by mechanically penetrating the plant and carrying the bacterium in with it (5).

This *Corynebacterium* is clearly a member of the *C. rathayi*, *C. agropyri*, and *C. tritici* group, which is characterized by yellow slime covering the inflorescences of the host, presence of a capsule, having a species of *Anguina* as vector, and occurrence on grasses. This particular species of *Corynebacterium* from annual rye grass has not been given a specific name because there is a lack of comparative information available for all the species in this group, and their taxonomy requires further consideration (5). However, the biochemical characteristics of the isolate described in this paper conform to the description of *Corynebacterium rathayi* by Cummins et al. in Bergey's Manual of Determinative Bacteriology (2) (J. Lloyd, *personal communication*).

One of the characteristics of the *Corynebacterium* - *Anguina* complex is the ability of these organisms to enter a hypobiotic state which enables them to withstand environmental extremes. Morphological changes in the fine structure of *Anguina tritici* associated with this state of latent life have been described (1). Changes, if any, in the morphology of *Corynebacterium*, associated with changes in its environment have not been described. Another feature of *Corynebacterium* - *Anguina* associations is the consistent occurrence of each species of *Anguina* with a particular species of *Corynebacterium*. The nature of this unique association between a nematode and a bacterium has not been examined. In this paper we describe differences in the comparative morphology of bacteria taken dry from bacterial galls and of those from agar cultures.

MATERIALS AND METHODS

The galled material examined in our experiments was obtained from both South Australia and Western Australia and the cultures were isolated from material obtained from Burra-Burra in South Australia and from Katanning in Western Australia.

Culture technique.—The yellow bacterial mass was squeezed out of the gall into sterile distilled water and a suspension was plated out on agar. These cultures contained other organisms which grew more quickly and masked the corynebacteria but, by repeated culturing of isolated material, we were able to isolate a pure Gram-positive strain which, when examined within 24 hr of plating out, formed as bright yellow colonies. The medium used was that described by Price (5), a modification of that developed by Kado and Heskett (3). It consisted of 10 g of sucrose, 8 g of casein hydrolyzate, 4 g of yeast extract, 2 g of KH_2PO_4, 0.3 g of $MgSO_4 \cdot 7\ H_2O$ and 15 g of agar made up to 1 liter with distilled water.

Light microscopy.—Dilute suspensions of bacteria were examined under a sealed coverslip using Nomarski differential interference contrast-, phase contrast-, and normal bright field optics.

Scanning electron microscopy.—A dilute suspension

of the bacteria was allowed to dry on a piece of coverslip stuck to an aluminium stub. These were coated with gold-palladium and observed under a Siemens Etec scanning electron microscope (SEM).

Transmission electron microscopy.—Bacterial material, whether from galls or cultures, was fixed in 3% glutaraldehyde at pH 7.3 in 0.15 M phosphate buffer for 1 hr at 5 C. Then it was washed twice by centrifugation in phosphate buffer and fixed for 1 hr at 22 C in 1% osmium tetroxide in 0.15 M phosphate buffer, pH 7.3. The material then was washed three times in phosphate buffer and transferred to Beam (Polaron Equipment Ltd., Watford, Hertfordshire, England) capsules in which it was serially dehydrated in 10, 20, 30, 50, 70, 80, 90% and absolute ethanol, with the material being separated at each step by centrifugation. Then it was placed in 1% uranyl acetate in absolute ethanol at 60 C for 18 hr followed by washing in absolute ethanol, treated with an ethanol:propylene oxide (1:1, v/v) mixture, followed by pure propylene oxide, and left in vacuo overnight in a propylene oxide:araldite (1:1, v/v) mixture. The material was finally polymerized in pure araldite at 60 C and sectioned with an LKB Ultratome fitted with glass knives. Some sections were stained with lead citrate for 10 min at 22 C. All sectioned material was observed under a Siemens Elmiskop 1A electron microscope operated at 80 KV.

RESULTS

One of the most definitive characteristics of these corynebacteria is their ability to adhere to the cuticle surface of a particular *Anguina* sp. (Fig. 1, 2). *Corynebacterium* sp. from *Lolium rigidum* has a characteristic shape that is perhaps shown most clearly when this organism is viewed with Nomarski interference optics (Fig. 4) or in the scanning electron microscope (Fig. 3). Its dimensions are approximately 0.6 - 0.75 × 1.5 μm. However, both larger and smaller specimens are common (Fig. 4) and some shrinkage occurs during dehydration and embedding (Fig. 5, 6, 7, and 8); thus, measurements obtained from sectioned material are slightly less than those from fresh material.

The capsule that surrounds each bacterial cell gives it a rounded appearance. This capsule is approximately 0.15-μm thick in sections of bacteria taken from dried galls (Fig. 7, 8) and is about 0.10-μm thick in sections of bacteria growing in cultures (Fig. 5, 6).

Random measurements of 25 sectioned bacteria taken from galls were compared with the same number of identically treated bacteria taken from cultures. The width of the bacterium without its capsule in the former case ranged from 0.2 μm to 0.4 μm and in the latter from 0.3 μm to 0.5 μm. Statistical analysis showed that there was a significant difference ($P < 0.001$) in the width of

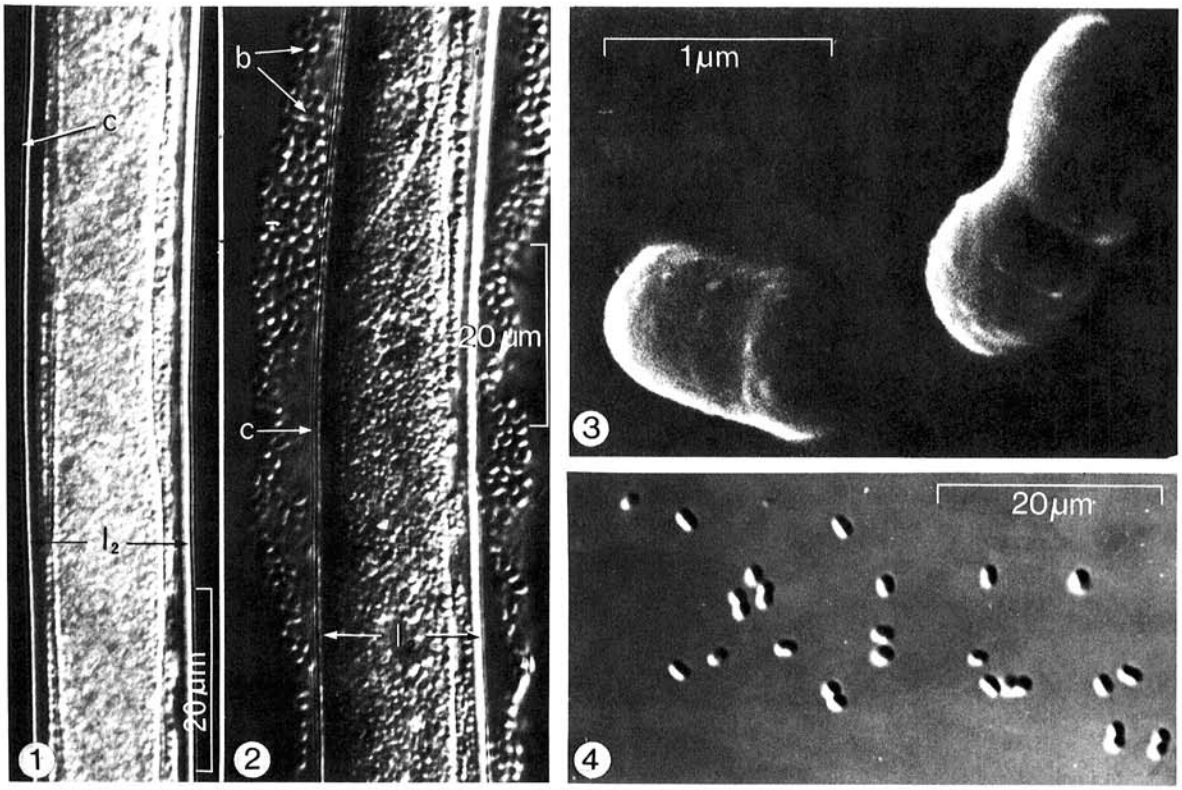

Fig. 1-4. 1) Part of a second-stage larva (l_2) of a bacteria-free *Anguina lolii* showing the absence of bacteria at the surface of the cuticle (c) (× 1,200). 2) Part of a l_2 of *Anguina lolii* that had been made to swim through a cell suspension of *Corynebacterium* sp. showing the bacteria (b) adhering to the surface of the cuticle (c) (× 1,200). 3) Cells of *Corynebacterium* sp. from annual rye grass (× 1,500). 4) Cells of *Corynebacterium* sp. from annual rye grass viewed with the scanning electron microscope (× 30,000).

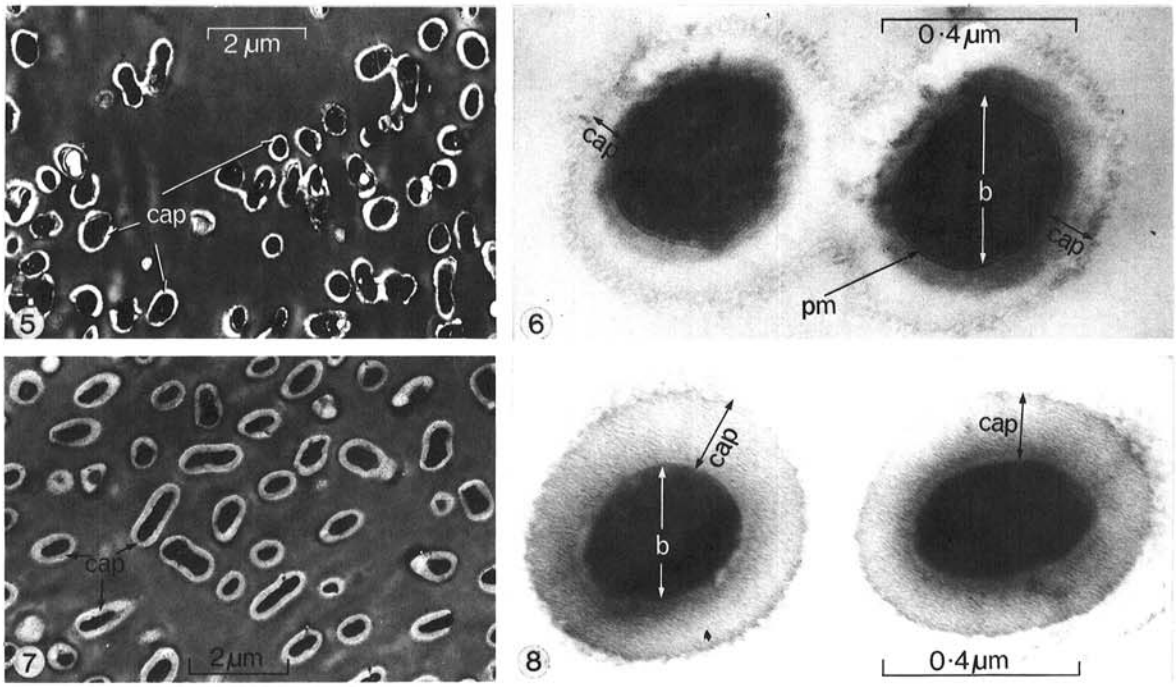

Fig. 5-8. 5) Section through cells of *Corynebacterium* sp. removed from culture media, showing the bacterium (b) and its surrounding capsule (cap) (× 6,500). 6) Section through a *Corynebacterium* cell removed from culture media, showing the hydrated bacterium (b), cell membrane (pm) and its surrounding capsule (cap) (× 65,000). 7) Section through cells of *Corynebacterium* removed from an annual rye grass gall and treated identically to that in Fig. 5. Shows the bacterium (b) and its surrounding capsule (cap) (× 6,500). 8) Section through a *Corynebacterium* cell removed from an annual rye grass gall and treated identically to that in Fig. 6. Shows the anhydrobiotic bacterium (b) and its surrounding capsule (cap) (× 65,000).

anhydrobiotic bacterial cells from galls (average width = 0.27 μm) and that of hydrated bacteria from cultures (average width = 0.39 μm). There was also a significant difference ($P < 0.001$) in the thicknesses of the capsules from these two sources (average anhydrobiotic width = 0.14 μm and average hydrated width = 0.08 μm). There was no significant difference in the overall dimensions of these bacteria and their capsules (average width in both instances = 0.55 μm). Presumably when the bacterium swells during hydration it does so at the expense of area previously occupied by capsule material.

Electron microscopic resolution of structures in the anhydrobiotic bacterium is difficult (Fig. 8). However, in hydrated forms a cell membrane can readily be detected surrounding the organism and separating it from the capsule (Fig. 6).

DISCUSSION

The adhesion of *Corynebacterium* sp. cells to the cuticle of *Anguina lolii* appears to involve an attractive force of considerable magnitude because several layers of bacteria can remain attached to the larva while it is actively moving. Some specific interaction between the surface membranes of the nematode and the capsule of the bacterium appears to have occurred. We find that noncapsulated bacteria such as *Escherichia coli* do not adhere to these nematodes.

The capsule also must have protective properties; corynebacteria are able to withstand dehydration associated with preparative techniques for the SEM better than the noncapsulated contaminant bacteria that we isolated. Furthermore, noncapsulated bacteria were much more easily damaged by the electron beam than similarly treated corynebacteria.

Vector nematodes of the genus *Anguina*, which share the same environment, and have the capacity to enter an anhydrobiotic state, also undergo some shrinkage in this condition (1). Both nematode and bacterium appear to be well adapted to withstand the hot and arid conditions of their summer environments.

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