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Cysts of *Azotobacter vinelandii* with Double Coats

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Cyst germination was studied in cultures of *Azotobacter vinelandii* ATCC 12837. The existence of cysts enclosed by double coats is described.

Members of the genus *Azotobacter* are characterized by their ability to form cysts. When encysted cells are placed in a favorable environment, they undergo a series of changes which transforms them to the vegetative form. Studies by Wyss et al. (6) and by Tchan et al. (4) have characterized the process of normal germination.

Cells of *A. vinelandii* ATCC 12837 were cultured and prepared for electron microscopy as previously described (1).

In the course of a previous study (1), large numbers of cysts of *A. vinelandii* were examined by electron microscopy. More than 96% of the cysts in liquid cultures germinated in what was considered a typical manner and gave rise to viable cells as determined by microscope observation and by plate counts. The remaining 4% or less was unresponsive to initial germination inducement, in that cyst morphology changed only slightly throughout the 24-hr incubation period. Of the cysts which failed to germinate in a normal manner in numerous cultures examined, a small number (0.04%) showed a distinctive morphology. Figure 1 shows what appears to be a typical germinating cyst. The central body enlarged and divided in the manner previously described (1). However, this cyst, apparently in the process of germination, is enclosed by a second intine-exine complex (IEx-IIn). The morphology of this cyst within a cyst is unambiguous. The apparent liberation of three cysts, one containing two central bodies, from a common exine (OEx) is shown in Fig. 2. The complex cyst in Fig. 2 appears to be the result of a cyst with a single central body which divided to give rise to a cyst with two central bodies, only one of which divided again prior to the formation of the inner exine (IEx). After deposition of the inner coat (IEx-IIn), the second body divided to form a cyst with two central bodies. We inferred from these micrographs that there are rare instances in cultures of germinating cysts of *A. vinelandii* when mature cysts, with complete coats, stop the normal germination sequence and initiate encystment. Consequently, these cysts become enclosed in a second intine-exine complex. Cysts with double coats are found as a part of the cyst population which fails to germinate in the first 24 hr in media supplemented with glucose.

Previous evidence has indicated that cyst germination, like endospore germination, could be halted but not reversed. The only tenable explanation for the data presented here resides in the assumption that the sequence of events which bring about encystment are closely related to the ones which bring about germination. The following data are offered as confirmatory evidence. (i) Intine vesicles are involved in the formation of the cyst coat (2); and (ii) Fig. 3 and 4 depict cysts in the process of germination and clearly show the presence of intine vesicles in germinating cysts. Intine vesicles observed in both encysting cells (2, 3, 5) and in germinating cysts (1) appear morphologically indistinguishable, and we suggest that they possess similar functions. On this basis, it appears that cysts with double coats could be formed if normal cysts in glucose-containing media produced intine vesicles in response to a germination stimulus, but for some reason (the aberrancy) the encystment process was set in motion and a second cyst coat (IEx-IIn) was deposited interior to the original cyst coat (OEx-OIn). Cysts with double coats have not been previously reported in *Azotobacter*, although they represent an interesting and important, but difficult to detect, phenomenon in these bacteria.

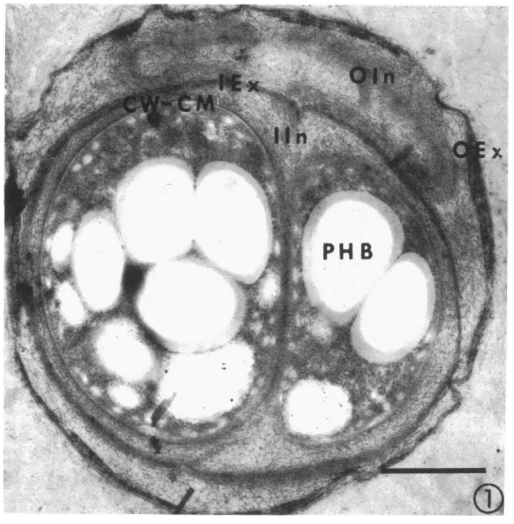

FIG. 1. *Thin-section of a cyst of A. vinelandii with two central bodies completely enclosed in a second cyst coat. Morphological structures of the cyst include the vegetative body cell wall-cell membrane (CW-CM), dispersed poly-β-hydroxybutyric acid granules (PHB), an inner intine (IIn), an inner exine (IEx), an outer intine (OIn), and an outer exine (OEx). Bar represents 1 μm.*

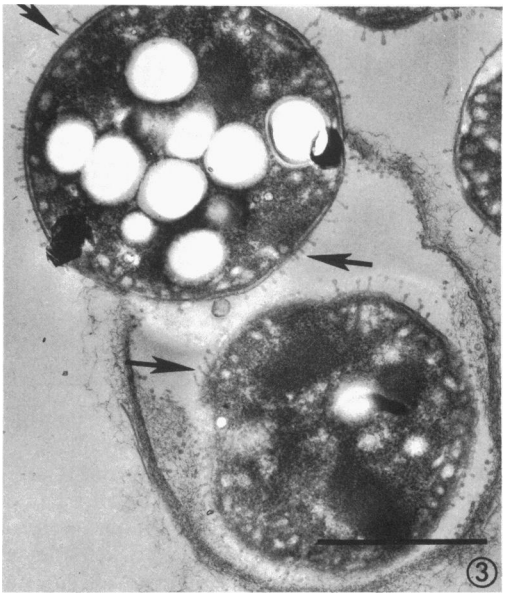

FIG. 3. *Thin-section of normal cyst in the process of germination. Intine vesicles are indicated (arrows). Bar represents 1 μm.*

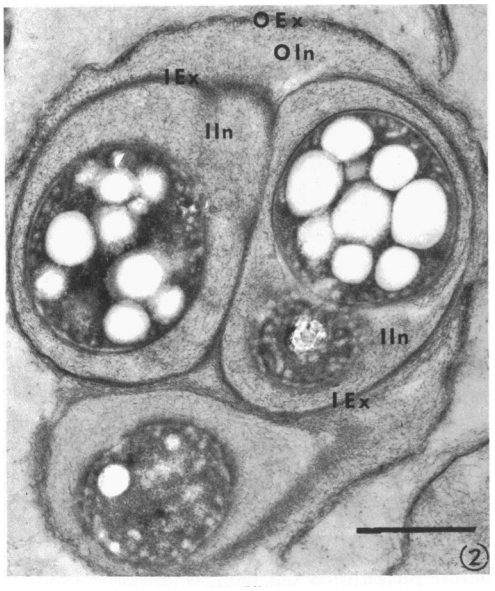

FIG. 2. *Electron micrograph of a complex structure of a cyst with a double coat in which the outer cyst coat (OEx-OIn) has been ruptured and three intact cysts, enclosed by a second coat (IEx-IIn), are emerging. Bar represents 1 μm.*

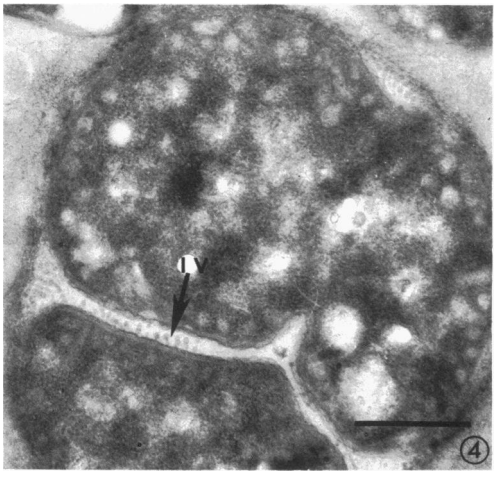

FIG. 4. *Thin section of a germinating cyst with multiple central bodies. Intine vesicles (IV) are observed at the surface of the cell wall-cell membrane of one of the central bodies. Bar represents 1 μm.*

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