Structure of the greening organism

KEYWORDS: Citrus, greening, bacteria, ultrastructure

Introduction
The greening disease of citrus in South Africa was described by Moll and Martin (1974), who suggested that this disease is caused by a prokaryote infecting the phloem cells of the citrus tree. This infection causes characteristic external symptoms of leaves and fruit as previously described (Moll and Martin, 1974).

It has already been determined, by observation of the organism in infected phloem, that the greening organism is a thin long rod with a distinct cell wall structure. However, placement into either Gram positive or Gram negative division is hindered by the unusual wall structure of these organisms. An electron dense peptidoglycan or murein layer typical of Gram negative, appears to be absent in the various greening isolates. However, recent evidence would suggest the inclusion of the greening organism as a Gram negative bacterium (Bové et al, 1980). Furthermore, Garnier and Bové (1983) recently described another morphological form in the infected citrus which they referred to as a ‘round form’.

In this work, a long rod bacterium was continuously isolated from infected citrus leaves, cultured and its ultrastructure studied.

Materials and methods
Organisms were isolated from greened citrus obtained from various regions in liquid MIG medium as described by Sibara (1982). Passed material was grown on solidified (1.5% agar) and liquid MIG medium. Colonies and liquid cultures were sampled and processed for electron microscopy.

Transmission electron microscopy:
The cells from liquid culture, washed free of all medium with 0.1 M Na-cacodylate buffer and colonies on agar plates were fixed in 2.5% glutaraldehyde, post fixed in 2% OsO4, stained for eight hours in 1% aqueous solution of uranyl acetate, dehydrated in series of alcohols and embedded in Epon/ Araldite. The dehydration and embedding were carried out in less than one hour, in order to prevent washing out of the uranyl acetate (Bové et al, 1980).

Ultra thin sections were cut on an LKB ultramicrotome and stained with uranyl acetate followed by a lead citrate. The sections were viewed on 100S Jeol electron microscope.

Scanning electron microscopy:
The cells were fixed overnight in 2.5% glutaraldehyde, post fixed in 2% OsO4 for one hour, dehydrated in series of alcohols, mounted on a glass cover slip; air dried and viewed on a Cambridge scanning electron microscope.

Results
Organisms isolated from several areas cultured in liquid medium, were found to be a long rod shaped bacteria (Fig. 1). They vary in length from 3,300-4,400 nm have a diameter of 300-450 nm, and are surrounded by a cell wall 25-30 nm in width. This cell wall

Fig 1

Fig 2

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showed the characteristic structure consisting of two double layers and an electron translucent zone between them (Fig. 2). The inner membrane appears more electrondense than the outer layer.

Scanning electron microscopy on cells taken from liquid cultures in log phase suggest that the cultured organism divides asymmetrically as depicted in Figs. 3, 4, 5. It appears that a small bud is formed, the bud growing until it is of similar size to parent cell.

Transmission electron microscopic studies of cells from similar cultures support this mode of division. A small bud is illustrated in Fig. 6 while in Fig. 7 the two daughter cells are symmetrical.

When growing the bacterium on solid medium small colonies were obtained. Very young colonies (two days of growth) were processed intact for TEM. At this stage of growth the cells throughout the colony appear to be long rods that were sectioned at different angles (Fig. 8). Dividing cells are abundant throughout sections of such young colonies. A section through a mature colony (five days old) reveals different morphological forms (Fig. 9). The growing cells on the surface of the colony are long rods, like the organism in log phase of liquid culture. These cells are arranged in a parallel fashion. However, towards the bottom of the colony most cells are round and of a larger width than in young colonies and demonstrate a more granular appearance. Sporulating cells were observed at the base of the mature colony (Fig. 9). The mature spore is released leaving a large round granular cell (Fig. 10). On studying stationary phase liquid cultures similar structures were observed (Fig. 11). SEM studies support the observation that the sporulating cells become shorter and wider (Fig. 12).

Discussion

The organism isolated from greened citrus demonstrated characteristic morphology, dimensions and cell wall structure when studied under optimal conditions as described by Moll and Martin (1974) in phloem cells of infected citrus.

The scanning and electron micrographs of dividing cells growing under optimal conditions suggests that the greening organism divides asymmetrically, starting with a small bud which enlarges gradually to its final size. Asymmetrical division of the greening organism was reported by Moll and Martin (1974) to occur within the infected plant phloem cells.

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Legends to Figures

Fig. 1.
A TEM micrograph of a longitudinal section through a greening organism grown in liquid culture.

Fig. 2.
The cell wall structure at a high magnification showing the characteristic two bilayers and the electron translucent zone between them.

Fig. 3.
Scanning electron micrograph of a budding cell grown in liquid culture.

Fig. 4.
Scanning electron micrograph of a more advanced stage of a budding cell grown in liquid culture.

Fig. 5.
Scanning electron micrograph of a late stage of a budding cell grown in liquid culture, where the two daughter cells are of equal length.

Fig. 6.
TEM micrograph of a budding cell grown in liquid culture.
Legend to Figures

Fig. 7.
TEM micrograph of a later stage of a budding cell where the two daughter cells are of equal length.

Fig. 8.
TEM micrograph of a section through a young colony (two days of growth) showing the growing long thin rods.

Fig. 9.
TEM micrograph of a section through a mature colony (five days old) showing the different morphological forms. The growing long rod cells on the surface of the colony and the large round cells at the bottom of the colony. Sporulating cells can be seen in the middle of the colony. Arrow indicating the direction of growth.

Fig. 10.
TEM micrograph of a spore being released from a large round cell.

Fig. 11.
TEM micrograph of a sporulating cell grown in liquid culture.

Fig. 12.
SEM micrograph of a sporulated cell in liquid culture.