SHORT ARTICLE

Electron microscopy studies on *Gardnerella vaginalis* grown in conventional and biofilm systems

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The cell-wall characteristics of *Gardnerella vaginalis* grown in conventional and biofilm systems were studied by electron microscopy. The gram-positive nature of the cell wall was confirmed. Novel cell-wall particles which appeared to be associated with cell division were also identified, particularly in organisms of biofilm origin.

*Gardnerella vaginalis* is considered to be an important entity in the aetiology of bacterial vaginosis. Recent work showing the relationship between the condition and an increased incidence in the delivery of pre-term infants, as well as the finding of an increased prevalence of HIV infection associated with the condition, have highlighted its importance [1,2]. Traditionally, *G. vaginalis* has been regarded as being a gram-variable organism on the basis of Gram's stain [3]. Electron microscopy studies have produced two opinions, with some workers stating that *G. vaginalis* is gram-positive, while others have stated that it is gram-negative. Reyn et al. studied the organism grown on solid media and considered it to be gram-positive [4]. This finding was supported by Sadhu et al., who studied four strains in broth culture [5]. The cell wall was shown to be typically gram-positive, being a homogeneous fibrillar structure with a usual thickness of 8–12 nm, but ranging up to 50 nm in thickness. Two other groups have stated that *G. vaginalis* is gram-negative, based on both electron microscopical and biochemical evidence [6,7]. A previous study showed that *G. vaginalis* grows to high concentrations in a continuous culture biofilm system with Sorbarod biofilms [8]. Thin sections of such biofilms studied by light microscopy have shown that the bacteria exist in microcolonies adherent to the cellulose matrix of the Sorbarod filter, in addition to individual adherent bacteria [9]. The present study examined the cell-wall characteristics of the organism grown on solid medium, in broth culture and in Sorbarod biofilms by electron microscopy, to determine if various growth conditions influence the cell-wall characteristics of *G. vaginalis*.

The strain of *G. vaginalis* (ATCC 14018), growth conditions and the Sorbarod biofilm system used were as described previously [8]. For electron microscopy studies the cells were harvested by centrifugation and resuspended in sodium cacodylate buffer containing glutaraldehyde 2.5%. This and all subsequent steps contained ruthenium red, at the concentrations stated previously [5]. Specimens were processed by standard electron microscopy procedures and then embedded in Araldite CY212 resin. Thin sections were produced with a Reichert Ultracut S microtome, stained with uranyl acetate and lead citrate and examined with a JOEL 100CX transmission electron microscope.

Fig. 1 shows the results of the electron microscopy studies. These essentially confirm the gram-positive nature of the cell wall; the overall range in cell-wall thickness was similar to that reported previously [5]. The gram-positive nature of the cell wall is clearly demonstrated in broth culture where the homogeneous fibrillar cell wall was clearly visible (Fig. 1a). The triple-layered plasma (cytoplasmic) membrane (7–8 nm thick) was clearly visible, as shown in sections of organisms from broth culture, solid medium and biofilm effluent (Fig. 1a, b and c). An electron-dense layer within the cell wall was also observed in sections of organisms (Fig. 1b and c). This structure most probably represents the collapse of hydrated cell-wall polymers to form the dense staining layer, a recognised feature of gram-positive bacteria [10].

Of particular interest were structures that were found rarely when the organism was grown in broth culture or on solid medium, but were more readily detected in
Fig. 1. Photomicrographs of thin sections of *G. vaginalis* examined by transmission electron microscopy. Sections shown were from bacteria grown: (a) in broth culture; (b) on solid medium; (c, d, e) biofilm effluent; (f) biofilm. p, plasma (cytoplasmic) membrane; e, electron-dense layer in cell wall; cwp, cell wall particle; s, developing septum. Bars represent 100 nm.
cells harvested from biofilm and biofilm effluent. These cell wall-associated particles have not been reported in previous studies of *G. vaginalis*. Their diameter was of the order of 18–20 nm. While variations in the number of these structures were seen, groups of seven identifiable ‘filaments’ above a thickened cell membrane were observed in organisms harvested from the biofilm effluent (Fig. 1d); these were also seen adjacent to one developing septum of a dividing cell (Fig. 1e). A complex arrangement of similar entities was also observed between two dividing cells, resembling mesosomal-like structures (Fig. 1f). It is of interest that similar structures have been noted with *Bacillus subtilis* [11], *Streptomyces* spp. [12] and certain species of mycobacteria [13, 14]. Some consider these entities to be precursor structures in the development of the cell-wall septum of gram-positive bacteria [11], or part of the mesosomal system. An overall model for the development of these structures has been proposed [13]. It has also been observed that these structures appear to develop on one side of the dividing cell [11], which was also observed here with *G. vaginalis*. (Fig. 1e). It is possible that the organised filaments in groups of seven seen here (Fig. 1d and e) may be the precursors to features reported previously [11–14].

We consider that the gram-positive nature of the cell wall clearly observed in this study confirms that *G. vaginalis* is solely a gram-positive organism. Any gram-variability in the nature of the cell wall can be ascribed to variations in the thickness of the cell wall as pointed out before [5]. The additional finding of structures that have been reported with true gram-positive bacteria and some mycobacterial species may be considered as confirmation of the gram-positive nature of this organism. The fact that these structures were more readily observed in the Sorbarod biofilm system shows that this simple technology may be very useful for determining the ultrastructure of bacteria in biofilm.

**References**