The Surface Layer of *Mycoplasma mobile* 163K and Its Possible Relevance to Cell Cohesion and Group Motility

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Mycoplasma mobile strain 163K tends to move in multicellular configurations, either as pairs or small groups of three or more cells, or as chain-like aggregations or microcolonies. Such wandering groups arise by transient association of independently moving cells. This behaviour of *M. mobile* was microscopically investigated and documented by sequences of microcinematographic pictures, as well as by photomicrographically recorded motility tracks. The presence of an extracellular slime layer was demonstrated in thin sections, by negative staining and by scanning electron microscopy. The possible association of this layer with the cohesive properties of the mycoplasma cells, enabling the formation of wandering groups, is discussed and a calculation of the magnitude of the cohesive force is provided.

INTRODUCTION

Mycoplasma mobile strain 163K, which was isolated from the gills of a freshwater fish (Kirchhoff *et al.*, 1983, 1987*b*; Kirchhoff & Rosengarten, 1984), is characterized by its gliding motility and its striking cell shape, with a pronounced head-like structure on the forward end (Kirchhoff & Rosengarten, 1984; Kirchhoff *et al.*, 1984, 1987*b*). It is distinguished from the other gliding mycoplasmas, i.e. *M. pneumoniae*, *M. genitalium*, *M. gallisepticum* and *M. pulmonis*, by the high speed of its movement on inert surfaces (Kirchhoff *et al.*, 1984; Rosengarten & Kirchhoff, 1987*a*) as well as on erythrocytes (Fischer *et al.*, 1987), by its ability to transport attached erythrocytes along a glass surface without significant speed reduction (Rosengarten *et al.*, 1987), and finally by its capability of chemotactic responses to a range of attractants (Kirchhoff *et al.*, 1987*a*). In a previous paper (Rosengarten & Kirchhoff, 1987*a*) we briefly reported that, in contrast to the other gliding mycoplasmas, *M. mobile* is able to form wandering groups ranging from pairs to multicellular configurations in which temporary intercellular associations are prevalent. These data, together with preliminary ultrastructural findings (Kirchhoff *et al.*, 1987*b*), suggested the presence of an extracellular mucus layer mediating the cohesion between the cells. In this paper, the tendency to multicellular behaviour of *M. mobile* is illustrated in more detail, and further evidence for the involvement of an extramembranous mucus layer in this process is presented.

METHODS

Organism and growth. *M. mobile* 163K (Kirchhoff & Rosengarten, 1984; Kirchhoff *et al.*, 1987*b*) was cultivated as previously described (Fischer *et al.*, 1987; Kirchhoff *et al.*, 1987*a*). The investigations were performed with the 14th to 22nd medium subculture after isolation.

Preparation for light microscopy. Organisms of *M. mobile* 163K were obtained by rinsing agar cultures showing dense growth with liquid culture medium. A 5 µl drop of the mycoplasma cell suspension, containing about 10⁸ c.f.u. ml⁻¹, was placed on a glass slide, covered with a glass coverslip and sealed with paraffin.

Dark-field microscopy and photomicrography. A Leitz standard photomicroscope equipped with dark-field optics was used. The movements of small groups of coherent cells were recorded photomicrographically by long

exposures, resulting in motility tracks on the film (Rosengarten & Kirchhoff, 1987*a*). Photomicrographs were taken with Kodak Tri-X Pan film (400 ASA) using exposure times of 10–40 s.

Interference microscopy and microcinematography. The interactions between the gliding mycoplasma cells were microcinematographically recorded with an Arri-Techno camera fitted to a Zeiss WL-microscope equipped with Normarski interference optics. Recordings were made on 35 mm colour film (ECH 5294) using a frequency of 24 frames s^{-1} .

Preparation for scanning and negative-contrast electron microscopy. All methods have been described previously (Kirchhoff *et al.*, 1987*b*). Briefly, *M. mobile* cells were grown in liquid medium on Pioloform (Dr Walter Hert Mikrotechnik, München, FRG) coated copper grids and glass coverslips for transmission and scanning electron microscopy, respectively. After 48 h, the attached cells were either negatively stained with ammonium molybdate or fixed in glutaraldehyde, postfixed with osmium tetroxide, dehydrated in ethanol, critical-point dried in liquid carbon dioxide and gold sputter-coated. The negatively stained specimens were examined in a Zeiss EM 10 at 80 kV, and the samples prepared for scanning electron microscopy in a Jeol JSM 35C at 25 kV.

Preparation for thin-section electron microscopy. For ultrastructural studies, the mycoplasma cells were grown in liquid medium for 48 h. The pelleting, washing, fixation, dehydration and embedding procedures have been described (Kirchhoff *et al.*, 1987*b*). In order to demonstrate a possible extramembranous mucus layer, the cells were fixed in the presence of ruthenium red (Luft, 1971) according to the method described by Springer & Roth (1973). Thin sections were cut on an LKB 4800 ultramicrotome with glass knives, stained with uranyl acetate followed by lead citrate, and examined in a Zeiss EM 10 at 80 kV (Kirchhoff *et al.*, 1987*b*).

Calculation of the cell cohesiveness. The cohesion force holding two adjacent cells together in an end-to-end position is equivalent to the force required to separate the two cells from each other. The magnitude of this force corresponds to the twofold value of the previously calculated propulsive force (Rosengarten *et al.*, 1987), which was deduced from the motility-driving energy (Rosengarten & Kirchhoff, 1987*b*; Rosengarten *et al.*, 1987) and depends on the medium viscosity, the geometrical cell properties and the gliding velocity (Rosengarten & Kirchhoff, 1987*b*).

RESULTS

Cell cohesion and group motility

Besides its movement as single cells, *M. mobile* showed a remarkable degree of cellular interactions during gliding, with a striking tendency towards multicellular groupings. This was observed particularly in dense cell populations, where the cells usually became slower (Rosengarten & Kirchhoff, 1987*a*) forming either small groups of two (Fig. 1), three (Fig. 2) or more (Fig. 3) cells aligned in parallel, or long linear chain-like aggregations (Fig. 4). These groups moved as single coherent units on the glass surface, behaving in this respect like the 'swarms' of many gliding bacteria (Reichenbach, 1981, 1984). In the extreme, the multicellular behaviour of *M. mobile* resulted in the translocation of microcolonies. The speed of movement of the smaller wandering groups was comparable to that of singly gliding cells, but the speed decreased with increasing size of the moving aggregate or microcolony. The intercellular interactions appeared to be rather loose, since the cell groupings constantly changed (Fig. 4): new groups formed, and existing groups enlarged, diminished or broke up. This behaviour correlated with the theoretically calculated strength of cohesion between two mycoplasma cells in an end-to-end position before separating and moving away from each other as individuals (Rosengarten & Kirchhoff, 1987*a*): by assuming a value of $46.05 \times 10^{-15} \mu J s^{-1}$ (Rosengarten & Kirchhoff, 1987*b*) for the propulsive energy of both dissociating mycoplasma cells, and $3.05 \mu m s^{-1}$ for the gliding velocity (Rosengarten & Kirchhoff, 1987*a, b*), the cohesion force holding the two cells together can be calculated to be approximately $3.0 \times 10^{-8} \mu N$, a value considerably less than the adhesion values calculated for the interaction of this organism with erythrocytes or glass (Rosengarten *et al.*, 1987).

Ruthenium-red-staining surface layer

The electron microscopic investigations provided strong evidence for the presence of an amorphous-to-floccular extracellular material coating the surface of the cytoplasmic membrane of *M. mobile* (Fig. 5*a*). The demonstration of an extramembranous layer surrounding the whole mycoplasma cell could be improved (Fig. 5*b*) by the use of an electron-dense osmium–ruthenium red complex which has an affinity for polyanions (Luft, 1971). The negative-contrast and

Figs 1, 2 and 3. Photomicrographs of sequential Nomarski interference-contrast microcinematographic frames (1 *a-b*, 2 *a-c*, 3 *a-c*) and negative prints of prolonged-exposure dark-field photomicrographs (1 *c*, 2 *d*, 3 *d*), showing the movements of small wandering groups of *M. mobile* 163K consisting of two (Fig. 1), three (Fig. 2) or four (Fig. 3) cells. Arrows indicate the resulting double (1 *c*) or triple (2 *d*) motility tracks and they point in the direction of translocation (1 *a-b*, 2 *a-c*, 3 *a-c*). Bars represent 10 μ m.

scanning electron micrographs of adjacent cells in Fig. 5(*c*) and Fig. 5(*d*), respectively, show that the extracellular polyanionic material can hold the cells together by bridging the interspace between them.

DISCUSSION

It has been assumed so far that gliding mycoplasmas move as individual cells only (Radestock & Bredt, 1977). There are, however, brief notes (Andrewes & Welch, 1946; Bredt & Radestock, 1977; Bredt, 1979) describing a kind of multicellular movement for *M. pulmonis* in that occasionally, nonmotile clustered cells were observed to be pulled by a motile cell. The motility studies in this and a preceding paper (Rosengarten & Kirchhoff, 1987*a*) show that, in contrast to *M. pneumoniae* (Radestock & Bredt, 1977; Bredt, 1979), *M. gallisepticum* (Erdmann, 1976; Bredt, 1979), *M. pulmonis* (Andrewes & Welch, 1946; Bredt & Radestock, 1977; Bredt, 1979) and *M. genitalium* (Taylor-Robinson & Bredt, 1983), *M. mobile* exhibits two distinct patterns of cell movement, namely single-cell movement and cell-group movement.

Fig. 4. A sequence of Nomarski interference-contrast microcinematographic pictures, showing the movement of a chain-like cell aggregate of *M. mobile* 163K. The leading cell within the chain is marked by a black arrow and the terminal cell by a white arrow. Bar represents 10 μ m.

Our investigations confirm previous observations on *M. mobile* ultrastructure (Kirchhoff *et al.*, 1987*b*) and gliding motility (Rosengarten & Kirchhoff, 1987*a*), namely that *M. mobile* possesses a surface layer of amorphous material external to the cell membrane (Kirchhoff *et al.*, 1987*b*) which may play an important role in the observed group motility (Rosengarten & Kirchhoff, 1987*a*). We have shown that this extramembranous material can be stained intensively with ruthenium red, which is thought by Luft (1971) to bind most strongly to extracellular polyanions, usually present as acidic mucopolysaccharides in glycoproteins. However, since ruthenium red does not react specifically with any group of chemical compounds, definite conclusions on the chemical nature of the *M. mobile* surface layer cannot be made.

Fig. 5. Transmission (*a*, *b*, *c*) and scanning (*d*) electron micrographs of both ruthenium-red-untreated (*a*) and ruthenium-red-treated (*b*) ultrathin-sectioned (*a*, *b*), negatively stained (*c*) and critical-point-dried (*d*) cells of *M. mobile* 163K, showing (*a*) a diffuse surface layer (SL) of (*b*) amorphous ruthenium red-staining material (RRSL) outside the cytoplasmic membrane (CM) which appears (*c*, *d*) to interconnect adjacent cells by forming thin intercellular bridges (arrows). Bars represent 0.1 μm (*a*, *b*) and 0.5 μm (*c*, *d*).

The presence of a ruthenium-red-staining surface layer has also been reported for the gliding mycoplasma species *M. pneumoniae* (Wilson & Collier, 1976) and *M. gallisepticum* (Ajufo & Whithear, 1978; Tajima *et al.*, 1982). Wilson & Collier (1976) suggested that the surface material of *M. pneumoniae* may play a role in its adhesiveness and its gliding motility. Indeed it is not unlikely that the polyanionic surface layer of the gliding mycoplasmas *M. pneumoniae*, *M. gallisepticum* and *M. mobile* has the same function as the extracellular slime of the gliding bacteria, namely (1) mediating attachment of the cell to the substratum (Marshall *et al.*, 1971; Marshall & Cruickshank, 1973) and (2) allowing and facilitating the movement on that substratum (Costerton *et al.*, 1961) by reducing the friction between the contacted surfaces. These properties are characteristic of a temporary adhesive (Humphrey *et al.*, 1979). Furthermore, the electron microscopic investigations of adjacent cells of *M. mobile* in this study have shown that the surface layer of this organism may also account for its cell cohesiveness and its ability to form multicellular wandering groups.

The tendency of the *M. mobile* cells to glide in groups with their long axes parallel and to remain in contact with adjacent cells resembles at least in some aspects the swarming

phenomenon observed in the gliding bacteria, especially in the myxobacteria (Reichenbach, 1981, 1984). However, in contrast to the genetically controlled motility of the myxobacteria (Hodgin & Kaiser, 1979*a, b*), the group motility of *M. mobile* obviously arises by transient association of primarily independently moving cells, or by cell multiplication leading to the formation of wandering microcolonies. Furthermore, in contrast to the organized social motility of the myxobacteria (Kaiser & Crosby, 1983), there is no coordination among the associated mycoplasma cells: they glide in a disorganized nonsocial manner. The aggregation of the *M. mobile* cells into wandering groups, particularly into long chains, appears to be related to physiological stress, such as nutrient deprivation, as indicated by reduction of motility, the phenomenon being most pronounced in dense cell populations (Rosengarten & Kirchhoff, 1987*a*).

The kind of interactions between cells that are necessary for producing a multicellular condition could have evolved from two mechanisms possessed by solitary cells, namely chemotaxis (Kühlwein & Reichenbach, 1965; Kaiser *et al.*, 1979; Burchard, 1984) and cell cohesion (Shimkets, 1986). *M. mobile*, exhibiting both chemotactic (Kirchhoff *et al.*, 1987*a*) and cohesive (Rosengarten & Kirchhoff, 1987*a*) properties, may represent one of the earliest evolutionary steps in this direction.

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