Ultrastructure and gliding motility of *Mycoplasma amphoriforme*, a possible human respiratory pathogen

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Despite their small size and reduced genomes, many mycoplasma cells have complex structures involved in virulence. *Mycoplasma pneumoniae* has served as a model for the study of virulence factors of a variety of mycoplasma species that cause disease in humans and animals. These cells feature an attachment organelle, which mediates cytoadherence and gliding motility and is required for virulence. An essential component of the architecture of the attachment organelle is an internal detergent-insoluble structure, the electron-dense core. Little information is known regarding its underlying mechanisms. *Mycoplasma amphoriforme*, a close relative of both *M. pneumoniae* and the avian pathogen *Mycoplasma gallisepticum*, is a recently discovered organism associated with chronic bronchitis in immunosuppressed individuals. This work describes both the ultrastructure of *M. amphoriforme* strain A39^T as visualized by scanning electron microscopy and the gliding motility characteristics of this organism on glass. Though externally resembling *M. gallisepticum*, *M. amphoriforme* cells were found to have a Triton X-100-insoluble structure similar to the *M. pneumoniae* electron-dense core but with different dimensions. *M. amphoriforme* also exhibited gliding motility using time-lapse microcinematography; its movement was slower than that of either *M. pneumoniae* or *M. gallisepticum*.

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INTRODUCTION

Within the genus *Mycoplasma* of the bacterial class *Mollicutes*, the *pneumoniae* group (Johansson & Pettersson, 2002) consists of numerous species, some commensal and some pathogenic, that live in association with vertebrates, including humans. *Mycoplasma amphoriforme* is a recently discovered member of the *Mycoplasma pneumoniae* cluster of the *pneumoniae* group (Webster *et al.*, 2003); its closest relatives include the avian respiratory pathogen *Mycoplasma gallisepticum* (Levisohn & Kleven, 2000), the human respiratory pathogen *Mycoplasma pneumoniae* (Waites & Talkington, 2004), and the human urogenital tract pathogen *Mycoplasma genitalium* (Jensen, 2004). Analysis of 16S rRNA sequence indicates a closer relationship with *M. gallisepticum* than with the others. *M. amphoriforme* strain A39^T was identified as a frequent inhabitant of the respiratory tracts of immunodeficient patients with chronic bronchitis, but not of immunocompetent patients (Webster *et al.*, 2003); it was cultured independently from such patients multiple times (Pitcher *et al.*, 2005). The details of the clinical case presented (Webster *et al.*, 2003) suggest that

M. amphoriforme is an opportunist that is likely pathogenic, especially in immunodeficient individuals.

The species of the *M. pneumoniae* cluster (Johansson & Pettersson, 2002) have numerous distinctive morphological features in common, including a flask-shaped appearance with a prosthecal polar structure, usually called the attachment organelle in *M. pneumoniae* literature and the terminal bleb in *M. gallisepticum* literature (Kirchhoff *et al.*, 1984; Balish & Krause, 2002). Best studied in *M. pneumoniae*, the attachment organelle is the primary site at which the mycoplasma cell attaches (cytoadheres) to the host cell using localized adhesin proteins (Feldner *et al.*, 1982; Hu *et al.*, 1982; Baseman *et al.*, 1987; Krause, 1996). The attachment organelle contains an electron-dense core (Biberfeld & Biberfeld, 1970), which is insoluble in the non-ionic detergent Triton X-100 (TX) (Meng & Pfister, 1980; Gobel *et al.*, 1981) and regarded as cytoskeletal. *M. pneumoniae*, *M. genitalium* and *M. gallisepticum* also exhibit gliding motility on surfaces (Kirchhoff, 1992); cells invariably glide in the direction of the attachment organelle (Bredt, 1968). Although the mechanism of gliding is not known, loss of virulence due to mutations in the attachment organelle adhesin protein P30 is associated with reduced speed in *M. pneumoniae* (Hasselbring *et al.*, 2005), suggesting that

Abbreviations: SEM, scanning electron microscopy; TN, Tris-NaCl buffer; TX, Triton X-100.

motility is a virulence-associated trait. Duplication of the attachment organelle is also linked with the cell-division process (Bredt, 1968; Seto *et al.*, 2001).

Cytadherence and virulence of *M. pneumoniae* depend upon a specific set of proteins associated with the attachment organelle, including those required for the presence of the electron-dense core (Balish & Krause, 2002; Seto & Miyata, 2003). Although ultrastructural aspects of the *M. genitalium* attachment organelle and the *M. gallisepticum* terminal bleb are less well-studied, loss of virulence in both species is associated with disruptions to homologues of these *M. pneumoniae* proteins (Mernaugh *et al.*, 1993; Dhandayuthapani *et al.*, 1999; Papazisi *et al.*, 2002; Mudahi-Orenstein *et al.*, 2003; May *et al.*, 2006). However, despite the work that has been done in *M. pneumoniae*, the relationship between the physical features of the attachment organelle and the processes of cytodherence, gliding motility, and attachment organelle duplication and cell division remains obscure.

Since *M. amphoriforme* is a possible human pathogen whose closest relatives include gliding mycoplasmas that have attachment organelles and associated cytoskeletal structures, we wanted to determine whether *M. amphoriforme* strain A39^T shared these virulence-associated features. We have used scanning electron microscopy (SEM) to investigate the morphological features of *M. amphoriforme*, identifying a probable homologue of the cytoskeleton-like electron-dense core of *M. pneumoniae* that is distinct from the cytoskeletal structures of *M. gallisepticum*, despite the external appearance of the cell resembling that of *M. gallisepticum* much more closely than that of *M. pneumoniae*. We have also characterized the gliding motility properties of *M. amphoriforme*. The data indicate that *M. amphoriforme* is characterized by a novel combination of morphological features of both *M. pneumoniae* and *M. gallisepticum*.

METHODS

Growth and culture conditions. *M. amphoriforme* strain A39^T (generously provided by J. Jensen, Statens Serum Institut, Copenhagen, Denmark) was grown in plastic tissue-culture flasks from frozen stocks for 4–6 days at 37 °C in SP-4 broth (Tully *et al.*, 1979) to mid-exponential phase (phenol red indicator was orange). Cells adhered to the plastic surface of the flask. For motility stocks, cells were prepared according to the method of Hasselbring *et al.* (2005), with minor modifications. Cells were grown to mid-exponential phase in 10 ml SP-4 broth. The supernatant was removed and the cells were scraped into 1 ml fresh SP-4 broth. This suspension was then dispensed into 50 µl aliquots and stored at –80 °C. *M. pneumoniae* strain M129 and *M. gallisepticum* strain R_{low} (generously provided by S. Geary, University of Connecticut, Storrs, CT, USA) were also cultured at 37 °C in SP-4 broth, with mid-exponential phase achieved after 2–3 days; motility stocks were prepared as for *M. amphoriforme*.

Time-lapse microcinematographic analysis. Thirty-five-microlitre samples of motility stock were inoculated into 765 µl SP-4 broth supplemented with 3% gelatin in individual chambers of four-well chamber slides (Nalge Nunc). The suspension was passed

seven times through a 25-gauge needle and incubated 3 h at the appropriate temperature. Cells attached to the slide were visualized using a Leica DM IRB inverted microscope equipped with a 100× objective. The sample was held at the appropriate temperature (see text) using a heating chamber. Phase-contrast images were captured at fixed intervals using a SPOT charge-coupled device camera and accompanying software (Diagnostic Instruments). Twenty-seven consecutive images were merged in different colour channels using Adobe Photoshop CS version 8.0, enabling visualization of cell movement. The initial image was magenta, the final image was yellow, and the merged image of all 27 frames was cyan. Immobile cells appeared black, whereas motile cells were in colour (see Fig. 5). The distance travelled by each cell corresponds to the length of the cyan line from the tip of the magenta cell to the tip of the yellow cell as measured using the SPOT software. The speed of each cell was computed by dividing the distance travelled by the duration of movement. Cells moving between fewer than 10 of the 27 frames were counted as motile but their speeds were not included in calculations.

SEM. Stocks were inoculated into 24-well plates containing 1 ml SP-4 broth and glass coverslips coated with 0.01% poly L-lysine. Cells were grown 1–3 days before processing. For analysis of TX-insoluble structures, coverslips were incubated 30 min at 37 °C in a solution containing 2% TX in Tris-NaCl buffer (TN; 20 mM Tris/HCl, pH 7.5/150 mM NaCl) (Stevens & Krause, 1991). For analysis of both whole cells and TX-insoluble structures, coverslips were rinsed in TN, 4×5 min and then fixed 30 min in 1% glutaraldehyde/2% formaldehyde/0.1 M sodium cacodylate, pH 7.2. Coverslips were subsequently rinsed with 0.1 M sodium cacodylate, 4×10 min, and dehydrated through a series of ethanol washes from 25% to 100%. After dehydration, the coverslips were critical-point dried and sputter-coated with 15 nm gold. Images were viewed and captured at the Electron Microscopy Facility at Miami University on a Zeiss Supra 35 FEG-VP scanning electron microscope operating at 5 kV. Dimensions of the TX-insoluble structures were measured using SPOT software.

RESULTS

The cellular morphology of *M. amphoriforme* is similar to that of *M. gallisepticum* but distinct from *M. pneumoniae*

Thin sections of *M. amphoriforme* observed by transmission electron microscopy suggest a flask-shaped morphology (Webster *et al.*, 2003; Pitcher *et al.*, 2005) consistent with related mycoplasma species of the pneumoniae group (Kirchhoff *et al.*, 1984; del Giudice *et al.*, 1985; Bradbury *et al.*, 1993). SEM of whole *M. amphoriforme* cells (Fig. 1a, b) confirmed that, like their relatives in the pneumoniae group, they are pleomorphic, with a flask shape. These images also revealed that in many cells the polar extension terminates in a single knob-like structure whose shape is like that of the terminal bleb of *M. gallisepticum* (Fig. 1c) but distinct from that of the *M. pneumoniae* attachment organelle (Fig. 1d). *M. pneumoniae* cells also consistently have a trailing filament (Fig. 1d); this was observed only very rarely in *M. gallisepticum* and *M. amphoriforme* (data not shown). Some *M. amphoriforme* cells appeared to be larger, with at least two extensions with knobs at opposite poles (Fig. 2a), closely resembling similar forms reported in *M. gallisepticum* (Morowitz & Maniloff, 1966). Others had

Fig. 1. Scanning electron micrographs of mycoplasma cells grown attached to coverslips. Images of cells are aligned with the attachment-organelle-like structure at the top. (a) *M. amphoriforme*; (b) *M. amphoriforme* cells with a single polar structure; (c) *M. gallisepticum*; (d) *M. pneumoniae*. Scale bar for (a), 1 μ m; scale bar for (b), (c) and (d), 250 nm.

three polar structures (Fig. 2b), and some cells appeared to have a pair of extensions in close proximity at one pole, occasionally with a third extension at the opposite pole (Fig. 2c).

M. amphoriforme* has TX-insoluble structures similar to those of *M. pneumoniae* but distinct from *M. gallisepticum

Glass-adherent cells of all three species were extracted in 2% TX. As observed by SEM, TX-insoluble structures of *M. amphoriforme* that remained adherent to the glass surface following extraction generally resembled those of *M. pneumoniae* (Fig. 3). These *M. amphoriforme* structures had elements resembling the terminal button, rod, base and fibres, though each element was larger, more consistently observed and better defined in *M. amphoriforme* than in *M. pneumoniae* (Fig. 3a). Fibres extending from the base were found in all *M. amphoriforme* specimens and exhibited considerable variability in orientation. Base-associated fibres were only rarely preserved in *M. pneumoniae* (Fig. 3b). The width of the rod-like portion of the *M. amphoriforme* TX-insoluble structure was 82 ± 15 nm (Table 1), nearly double the width of the *M. pneumoniae* rod. The total length of the rod and the approximately spherical, terminal button-like structure was 254 ± 20 nm in *M. amphoriforme* (Table 1), slightly longer than we measured for *M. pneumoniae*. The base in the *M. amphoriforme*

Fig. 2. Scanning electron micrographs of presumptively dividing *M. amphoriforme* cells grown attached to coverslips. (a) Cells with attachment organelle-like structures at opposite poles; (b) cells with three protrusions roughly equidistant; (c) cells with adjacent protrusions. Scale bar, 250 nm.

structure was irregular but roughly rectangular, 164 ± 16 nm wide and 90 ± 16 nm long (Table 1), substantially larger than that of *M. pneumoniae* (Fig. 3). Despite the close phylogenetic relationship with *M. amphoriforme* and *M. pneumoniae*, the TX-insoluble fraction of *M. gallisepticum* was entirely dissimilar, with filamentous or fibrous masses lacking obvious organization and occupying the volume of the entire cell (Fig. 3c).

In *M. pneumoniae*, the two parallel components of the rod have been observed distinctly only in an *hmw3* mutant (Willby & Krause, 2002) and in cross-section (Hegermann *et al.*, 2002). In contrast, images of the TX-insoluble structures of *M. amphoriforme* clearly revealed that some rods were approximately doubled in width with a longitudinal cleft, suggestive of a double rod extending from a single base (Fig. 3a, images 4–6). Furthermore, in some cases, the rods were partly (Fig. 3a, image 7) or completely (image 8) separated, though still apparently extending from a single base, conceivably corresponding to cells with two extensions at a single pole (see Fig. 2c). In *M. pneumoniae* this cleft was clearly observed only rarely (Fig. 3b, image 7).

Fig. 3. Scanning electron micrographs of mycoplasma cytoskeletal structures. *M. amphoriforme* (a), *M. pneumoniae* (b) and *M. gallisepticum* (c) cells were grown attached to coverslips and extracted with 2 % TX (see Methods). T, terminal button; R, rod; B, base; F, fibrous extensions. Arrows indicate longitudinal clefts; carets indicate individual fibres. Scale bars: (a) and (b), 250 nm; (c), 1 µm.

***M. amphoriforme* cells exhibit slow gliding motility**

Since SEM images indicated that *M. amphoriforme* has a combination of ultrastructural features of *M. pneumoniae* and *M. gallisepticum*, both of which exhibit gliding

Table 1. Dimensions of the cytoskeleton-like structure of *M. amphoriforme* and *M. pneumoniae*

Measurements (in nm, mean ± SD) taken from 29 representative structures for *M. amphoriforme* and 34 representative structures for *M. pneumoniae* (see Fig. 3). ND, Not determined due to inconsistent resolution of the boundary between the terminal button and the rod.

	<i>M. amphoriforme</i>	<i>M. pneumoniae</i>
Terminal button width	88 ± 12	44 ± 10
Terminal button length	73 ± 12	ND
Rod width	82 ± 15	45 ± 8
Rod length	181 ± 17	ND
Base width	164 ± 16	64 ± 14
Base length	90 ± 16	56 ± 14
(Terminal button + rod) length	254 ± 20	225 ± 20
Total length*	343 ± 23	282 ± 25

*Excluding the fibres.

motility (Bredt, 1979), we investigated gliding motility in *M. amphoriforme*. Consecutive phase-contrast images of individual fields revealed that individual *M. amphoriforme* cells glided (Fig. 4). Although the polar protrusion (see Fig. 1) was difficult to see clearly at this resolution, it appeared that cells were moving in the direction of a tapered pole, consistent with this structure (Fig. 4, arrow-head). Cells moved in paths that were clockwise, counter-clockwise and approximately straight, and also changed directions (data not shown).

Snapshots of living cells attached to glass under previously established optimal conditions for motility (Hasselbring *et al.*, 2005) were captured at regular intervals; the paths taken by individual cells were characterized with respect to velocity and analysed as a population. We used a merged image of 27 consecutive frames captured at intervals adjusted to ensure that consecutive images of a given cell were overlapping. The false-coloured merged image was overlaid with false-coloured images of the first and last frames in order to indicate unambiguously the starting and stopping points for each cell; as a result, immotile cells were black in the final image, whereas motile ones appeared in colour (see Fig. 5). Finally, the length of the path between the tip of the starting image and the final image was measured.

As a positive control, motility of *M. pneumoniae* at 37 °C was measured at intervals of 1 s at a mean speed of

Fig. 4. Consecutive phase-contrast images of *M. amphoriforme* gliding motility at 5 s intervals at 37 °C. Three representative cells are indicated by arrows orientated horizontally, vertically and diagonally. In the first frame (0 s), the carets point to the tapered ends of the indicated cells. In the last frame (40 s), the asterisks indicate the positions of each of the indicated cells at 0 s. Scale bar, 1 μ m.

$336 \pm 59 \text{ nm s}^{-1}$ (Table 2), with 51 % of cells gliding at a given time, consistent with previous results (Radestock & Bredt, 1977; Hasselbring *et al.*, 2005; Seto *et al.*, 2005a). As measured at 37 °C using 2 s intervals (Table 2), *M.*

gallisepticum strain R_{low} glided with a mean speed of $131 \pm 38 \text{ nm s}^{-1}$, faster than reported previously for *M. gallisepticum* (Bredt, 1979), with 64 % gliding at a time. Multiple fields of gliding *M. amphoriforme* cells were

Table 2. Gliding motility parameters

Species	Total no. of cells*	No. of motile cells	Percentage of cells moving per frame†	Mean speed (nm s ⁻¹)‡	Range of speeds (nm s ⁻¹)	Mean speed of field (nm s ⁻¹)§
<i>M. amphoriforme</i>	1034	621	53	49 ± 19	15–133	48 ± 4
<i>M. gallisepticum</i>	421	278	64	131 ± 38	50–286	131 ± 3
<i>M. pneumoniae</i>	149	80	51	336 ± 59	197–538	335 ± 8

*Total number of motile cells in all fields.

†Because different populations of cells are motile in different frames, the total number of motile cells over an observation period is greater than the total number of motile cells in any one frame.

‡Mean speed of motile cells $\pm$ SD.

§Mean of mean speeds of motile cells in each field counted $\pm$ SD.

Fig. 5. Measurement of *M. amphoriforme* gliding speed by time-lapse microcinematographic analysis. Phase-contrast images of cells attached to glass slides held at 37 °C were captured at 5 s intervals. Twenty-seven consecutive images were merged and the resulting track was measured (see Methods). (a) Image at 0 s; (b) image at 130 s; (c) merged image of all 27 frames between 0 s and 130 s; (d) false-coloured overlay of panels (a) (magenta), (b) (yellow) and (c) (cyan). See Methods for details. For each cell, the distance was measured from the leading end of the magenta image to that of the yellow image along the cyan path, the number of frames in which the cell had moved was determined, and the speed was calculated. Scale bar, 1 μ m.

Fig. 6. Distribution of mycoplasma velocities about the mean. Gliding speeds of the individual motile cells for which data are shown in Table 2 were grouped into bins of $0.2 \times$ the mean, designated x , for each species. The percentage of cells with speeds in a given range out of the total population of motile cells was plotted against the speed as a fraction of the mean speed for each species. White bars, *M. amphoriforme* ($x=49$); black bars, *M. gallisepticum* ($x=131$); grey bars, *M. pneumoniae* ($x=336$).

captured at 5 s intervals and analysed in this manner (Fig. 5). At 37 °C, *M. amphoriforme* cells glided at $49 \pm 19 \text{ nm s}^{-1}$, with 53 % of cells moving at any instant (Table 2). At room temperature *M. amphoriforme* cells attached poorly and moved more slowly; among those cells that did attach to the glass surface, a substantially smaller fraction was motile (data not shown).

The large standard deviation in the speed of *M. pneumoniae* (Table 2) was distinct from that reported by previous workers (Hasselbring *et al.*, 2005). However, for each species, gliding rates were unimodal, with a substantial proportion of cells gliding up to twofold faster than the mean speed (Fig. 6). The relatively low standard deviation of mean velocities from field to field for each species (Table 2) indicates that the range of cell velocities is present in each field, as opposed to conditions varying during analysis of each field.

DISCUSSION

Mycoplasma virulence is multifactorial, with contributions from activities associated with adherence (Balish & Krause, 2002), competition with host cells for nutrients (Razin *et al.*, 1998), production of harmful molecules such as peroxide (Tryon & Baseman, 1992), phase variation of surface molecules (Yogev *et al.*, 2002) and motility (Miyata, 2005), in addition to immunopathological factors (Simecka, 2005). The molecular bases for these traits vary among mycoplasma species and are largely not well understood. In the human pathogen *M. pneumoniae*, molecules and cellular structures associated with cytodherence have been identified, including adhesins and cytodherence accessory proteins, all of which appear to be involved in formation and function of a polar protrusion, the attachment organelle (Krause & Balish, 2004), which is also instrumental in gliding motility. *M. gallisepticum* also has such a structure, albeit with a somewhat different appearance, and possesses related molecules, as do other species of the *M. pneumoniae* cluster (Balish & Krause, 2005), suggesting that this cluster of related species uses a common set of virulence factors. However, these factors have been well studied only in *M. pneumoniae* to date, and our understanding of them is limited. Elucidating correlations between attachment organelle-associated characteristics and virulence-associated properties of species other than *M. pneumoniae* is essential for understanding the common molecular underpinnings of virulence in species of the *M. pneumoniae* cluster. At the same time, differences identified among these species will underscore specific adaptations of each, casting crucial light on the disease process itself. *M. amphoriforme*, a likely human pathogen (Webster *et al.*, 2003; Pitcher *et al.*, 2005), is a particularly relevant choice for such studies.

Our results indicate that in contrast to the notion that all of these related mycoplasma species cause disease in essentially the same way at the cellular level, *M. amphoriforme* has a novel combination of the virulence-associated features

of its relatives. *M. amphoriforme* cells resembled those of *M. gallisepticum*, not *M. pneumoniae*, in overall morphology, including the absence of a trailing filament and the presence of a short polar protrusion terminating in a widened knob. In striking contrast, *M. amphoriforme* cells had discrete TX-insoluble structures with significant resemblance to the *M. pneumoniae* electron-dense core, which is essential for the architecture and functions of the attachment organelle in cytodherence and gliding motility (Balish & Krause, 2002). A similar TX-insoluble structure was not distinguishable in *M. gallisepticum* cells treated in a like manner amidst a remarkably complex mass of material (Fig. 3); extraction with concentrations of TX up to 10 % did not substantially alter the appearance of the fraction (data not shown). These results do not discount the possibility of such an element being present within the *M. gallisepticum* TX-insoluble fraction, but they demonstrate that a range of ultrastructural complexity not previously anticipated is present among the species of the *M. pneumoniae* cluster. Although similarities to related species make it likely that the polar structure of *M. amphoriforme* is likely to be involved in infection, its role remains to be determined.

We propose that, as in *M. gallisepticum*, the polar protrusions observed in *M. amphoriforme* (Figs 1 and 2) function as sites of attachment, though this remains to be demonstrated formally; however, the contribution to this structure by TX-insoluble cell components is likely to be more similar to *M. pneumoniae* than to *M. gallisepticum*. Even so, the *M. amphoriforme* TX-insoluble structure is nearly twice as wide as and slightly longer than that of *M. pneumoniae*, with a much more prominent terminal button and base (Fig. 3); this difference in ultrastructure might point towards differences in properties associated with attachment organelle function, though it is not yet possible to propose the specific relationship between structural and functional differences. Like *M. pneumoniae*, *M. amphoriforme* has a set of large TX-insoluble proteins (data not shown); future studies will address the identities, locations and cellular roles of these proteins.

Two observations concerning the ultrastructure of the *M. amphoriforme* TX-insoluble structure relate to proposed roles of the electron-dense core in attachment organelle duplication in *M. pneumoniae*. First, the *M. amphoriforme* TX-insoluble structure commonly revealed a cleft within the rod portion (Fig. 3), its visualization perhaps facilitated by the greater width of the rod as compared with *M. pneumoniae*. This cleft might delimit the two parallel elements of the *M. pneumoniae* core (Hegermann *et al.*, 2002); it also might represent a feature unique to *M. amphoriforme* or, at any rate, one that has not been described in *M. pneumoniae*. Second, it was possible to see occasional *M. amphoriforme* images in which a base had two separate and apparently single rods emanating from it (Fig. 3a, images 7 and 8). A possible interpretation of these two observations is that the electron-dense core in

both *M. pneumoniae* and *M. amphoriforme* consists of a paired rod whose two parallel elements separate longitudinally during attachment organelle duplication, as has been proposed (Krause & Balish, 2004). Alternatively, the new rod might be synthesized *de novo* on the same base as the original rod, with division occurring at the base. It is unclear why identical treatment of *M. pneumoniae* and *M. amphoriforme* results in differential preservation of these presumably homologous structures; perhaps the interaction between the base and the rod is stronger in *M. amphoriforme*, or this structure might be differentially TX-insoluble in the two species. Regardless, because duplication of attachment organelles and migration to the opposite pole in stages is postulated as the means of *M. pneumoniae* cell division (Bredt, 1968; Seto *et al.*, 2001), images of relatively large *M. amphoriforme* cells with two or three protrusions (Fig. 2) reinforce the idea that duplication of the organelle and cell division are linked.

In addition to cytodherence and cell division, a third aspect of mycoplasma physiology involving the attachment organelle is gliding motility. In *M. pneumoniae* as well as in *Mycoplasma pulmonis* and *Mycoplasma mobile*, which also exhibit polar protrusions despite lacking homologues of attachment organelle components, the polar structure leads the cell during gliding motility (Miyata, 2005). A handful of novel proteins has been directly implicated in gliding in these species, with an ATP-dependent attach-and-release mechanism suggested (Hasselbring *et al.*, 2005; Seto *et al.*, 2005a, b; Uenoyama & Miyata, 2005). Despite the fact that the polar structure of *M. amphoriforme* is only marginally resolvable by light microscopy, time-lapse microcinematographic images of glass-adherent *M. amphoriforme* cells suggested that, as in the other species, gliding occurs in the direction of a tapered pole (Fig. 4). However, the mean speed was some sevenfold slower than that of *M. pneumoniae* and nearly threefold slower than that of *M. gallisepticum* (Table 2). Neither the molecular basis for gliding motility nor its physiological role is clear, but its conservation in species with different characteristics is further evidence that it is important.

In conclusion, *M. amphoriforme*, a recently discovered likely respiratory pathogen of the immunosuppressed, has virulence-associated ultrastructural features in common with the related species *M. pneumoniae* and *M. gallisepticum*. However, these features are present in a unique combination, the overall morphology resembling the latter and the internal structure resembling the former. The dimensions of the *M. amphoriforme* TX-insoluble structure, which is a probable component of an attachment organelle, differ distinctly from those of the comparable structure in *M. pneumoniae*. *M. amphoriforme* also exhibits gliding motility, with a speed that is slower than either of the other species. The presence of distinct varieties and combinations of established virulence characteristics from other species underscores the fact that an understanding of *M. amphoriforme*-associated disease must derive from

direct characterization of this organism, not just from application of information concerning its relatives.

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