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Surface motility of *Xylella fastidiosa* visualized by oblique illumination

J. Chen, R. Groves, E. Civerolo, and S. Livingston

Abstract: Stereomicroscopic observations using oblique illuminations revealed the presence of two types of movement trails by *Xylella fastidiosa* strains (A- and G-genotypes) isolated from almond-leaf scorch samples on the surface of PW and PD3 culture media. The A-genotype strains showed curved motility trails, and the G-genotype strains showed straight motility trails. Haloes were found around some G-genotype colonies due to the excretion of unknown factors and (or) compounds, which might be related to bacterial surface motility.

Key words: Xylella fastidiosa, surface motility, slime trail, almond-leaf scorch.

Résumé : Des observations par illumination oblique en stéréomicroscopie ont révélé la présence de deux types de traces de mouvement laissées par des souches de *Xylella fastidiosa* (génotypes A et G) isolées d'échantillons de brûlure foliaire de l'amandier, à la surface des milieux de culture PW et PD3. Les souches provenant du génotype A montraient des traces de mouvement incurvées alors que les souches du génotype G montraient des traces de mouvement droites. Des halos ont été trouvés autour des colonies du génotype G, dus à l'excrétion de facteurs ou (et) de composés inconnus qui pourraient être liés à la motilité bactérienne de surface.

Mots-clés : Xylella fastidiosa, motilité à la surface, trace de mucus, brûlure foliaire de l'amandier.

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Xylella fastidiosa is a bacterial pathogen of many economically important crops, such as grape, almond, and citrus. Because of the difficulty of in vitro cultivation, many biologic traits of this bacterium are not well understood. Among them is surface motility, a biologic feature related to the pathogen–environment interaction, such as biofilm formation (Costerton et al. 1995; Merz and Forest 2002). In the original description, Wells et al. (1987) described *X. fastidiosa* as nonmotile. Type IV pili-dependent twitching motility in *X. fastidiosa* was recently reported by Meng et al. (2005). To further study this bacterial behavior, we examined the surface movement of *X. fastidiosa* on two common media (PW-G and PD3-G). The former was derived from PW medium (Davis et al. 1981*a*), and the latter was derived from PD3 medium (Davis et al. 1981*b*) and solidified by GelRite (Hill and Russell 1995).

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Two *X. fastidiosa* strains (M12 and M23) isolated from almond trees affected with almond-leaf scorch disease (ALSD) in Kern County in the San Joaquin Valley of California in 2003 were initially used. M12 is an A-genotype, causing ALSD only, and M23 is a G-genotype, causing both grape Pierce's disease (PD) and ALSD (Chen et al. 2005). Another 32 A-genotype and 54 G-genotype strains from the San Joaquin Valley were subsequently examined. Bacterial cultures were streaked to single colonies. The culture plates were sealed with Parafilm and incubated at 28 °C for up to 50 days. Bacterial colonies were examined from inverted plates, using a Leica S8AP0 stereo-binocular microscope (Leica Microsystems Inc., Bannockburn, Illinois) with a maximum magnification of × 80. A flexible fiber-optic light was used for illumination at an oblique angle of ~45°. The observation was focused on the medium surface where the bacterial colony expanded. Images were recorded in TIFF format (1920 × 2560 pixels), using a LEICA DFC 480 digital camera.

Curved-type movement trails in A-genotype strains

Movement trails were observed in >99% of the colonies of strain M12 on both PW-G and PD3-G (Figs. 1A and 1B). The movement trails were characteristically nonstraight or curved (CMTs), indicating a constant change in direction. The mean diameter was ~4.0 μm, measured from enlarged images on a computer monitor. With a cell size of 0.25 to 0.35 μm by 0.9 to 3.5 μm (Wells et al. 1987), a CMT could

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Fig. 1. Movement trails from *Xylella fastidiosa* almond-leaf scorch strains 8 days after inoculation. PW-G (A) and PD3-G (B) media show curved movement trails from A-genotype strain M12; PW-G (C) and PD3-G (D) media show no visible movement trails from strain M12S, a spontaneous mutant of strain M12; PW-G (E) and PD3-G (F) media show straight movement trails and haloes (arrows) from G-genotype strain M23. Scale bar = 500 μm.

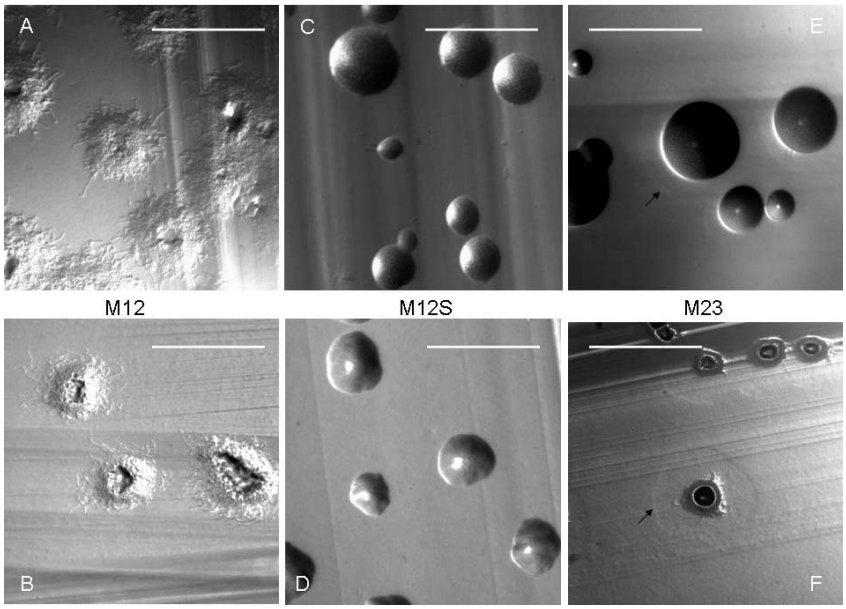
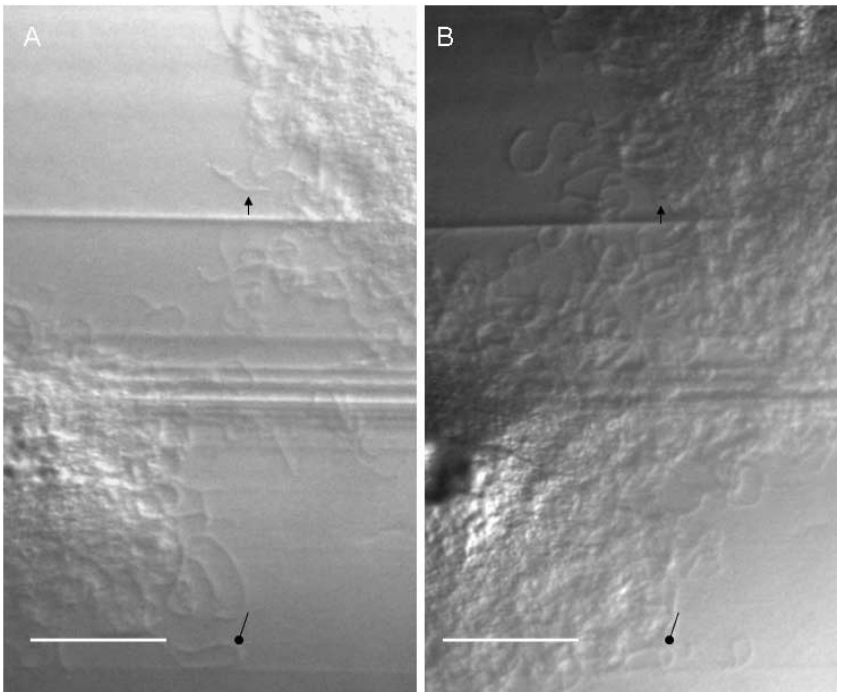


Fig. 2. Expansion of curved motility trails of *Xylella fastidiosa* M12 in 24 h. A, day 6; B, day 7. Arrows and dots identify the same positions. Scale bar = 200 μm.



be made by a maximum of 16 (4.0 μm/0.25 μm = 16) bacterial cells. The trails extended >200 μm in 24 h (Figs. 2A and 2B). This is 57 times the size of *X. fastidiosa* cells (200 μm/3.5 μm = 57). With a doubling time of 0.9–3.5 days (Wells et al. 1987), CMTs were not formed because of the concatenation of bacterial cells. All of the 32 A-genotypes strains exhibited CMTs, as shown in Fig. 3.

CMTs were seen as early as 3 days after inoculation (Fig. 3A), an early event in the bacterial growth. The bacterial cells could move independently, across grooves created by the inoculation loop, to a distance of 500 μm (Fig. 3A). Microsatellite colonies formed as CMTs expanded, giving rise to the "rough" colony morphology (Fig. 3B) reported earlier (Chen et al. 2005). As slime or exopolysaccharide ac-

Fig. 3. Curved movement trails and colony morphology in different *Xylella fastidiosa* A-genotype strains. A, strain R45; B, strain R22; C, strain R59; and D, strain R37. Scale bar = 500 μm.

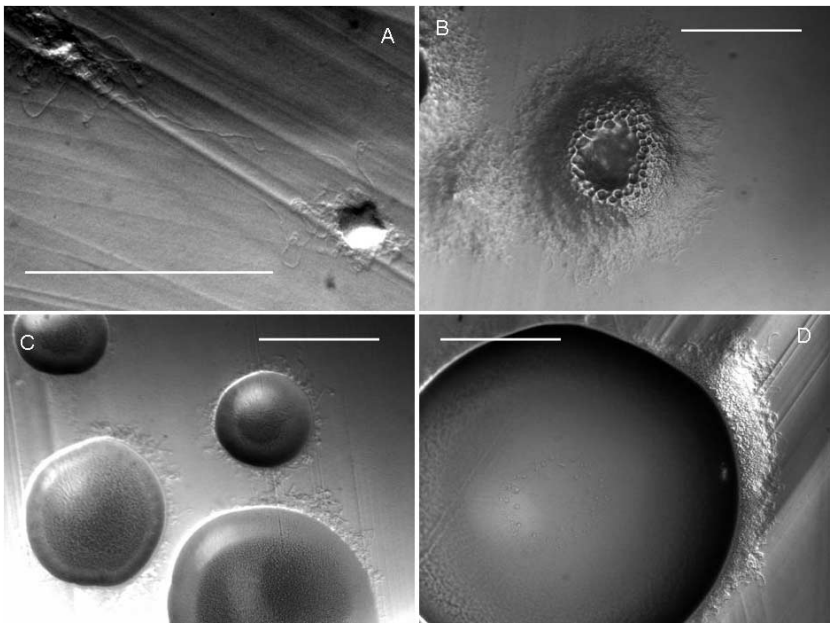
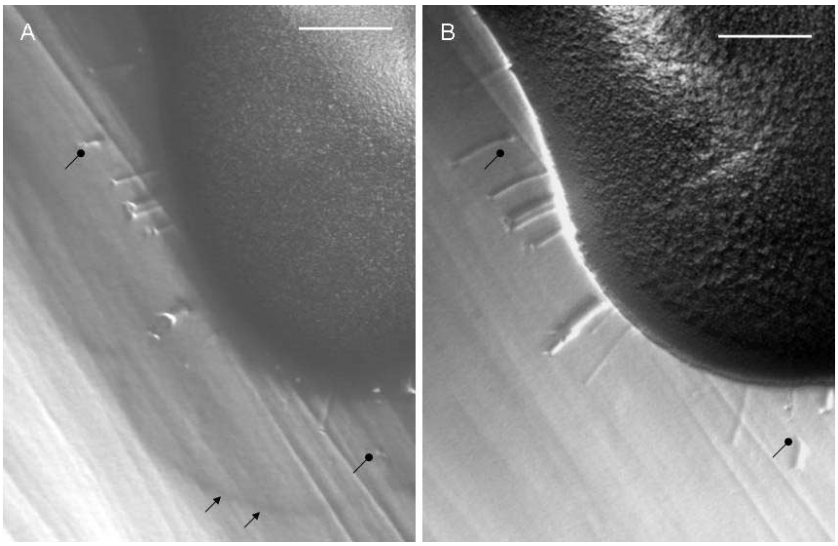


Fig. 4. Expansion of straight motility trails of *Xylella fastidiosa* M23 in 24 h. A, day 6; B, day 7. Dots identify the same positions. Arrows indicate the presence of a halo at day 6 and but not at day 7. Scale bar = 200 μm.



cumulated, the bacterial colony became "smoother", but CMTs remained observable at the margins (Figs. 3C and 3D).

Almeida and Purcell (2003) reported that the ALSD Dixon-like strain (A-genotype) did not grow in PD3 medium. We observed that the bacterium survived and moved around for a period of time on PD3-G when directly transferred from PW-G (Fig. 1B), although the bacterium did not survive three subculture passages on PD3-G. If PD3 medium is considered to be nutritionally poorer than PW medium, then the presence of CMTs on PD3 indicates that *X. fastidiosa* cells remained motile under a nutritionally stressed environment.

During the subculturing of M12 on PW-G, a spontaneous CMT$^-$ mutant, M12S, was isolated (Figs. 1C and 1D). The strain was subcultured over 20 passages, and no CMT reversion occurred. This presents an irreversible phase variation (Van der Woude and Baumler 2004). The mutant will be useful for further study of genetic determinant(s). Like the wild-type strain, M12S grew on PD3-G (Fig. 1D) but did not survive three sequential subcultures.

Straight-type movement trails in G-genotype strains

Strain M23 exhibited a smooth colony morphotype on PW-G (Fig. 1E) (Chen et al. 2005). Careful examination, however, showed straight movement trails (SMTs), ~8 μm in diameter (Figs. 1E, 1F, 4A, and 4B), at a low frequency of 0.8% (from 1000-colony examination). The low frequency of SMTs was also observed in the 54 G-genotype strains. On PD3-G, SMTs were found in up to 50% of the

colonies at an early stage (e.g., day 3), probably because of the slower slime production on the medium.

The straight-line nature (Figs. 4A and 4B) indicated that the bacterial SMT had a defined direction. SMTs could expand >110 μm within 24 h (Figs. 4A and 4B). This is 31 times the length of a *X. fastidoisa* cell (110 μm/3.5 μm = 31), excluding the possibility that SMTs were concatenates of bacterial cells with the fastest doubling time of 0.9 days (Wells et al. 1987). As shown in Figs. 4A and 4B, some SMTs are discontinuous. One possible explanation is that the bacteria initiated a movement without SMT production, and then switched to SMT motility. Exopolysaccharide accumulation, in most cases, covered the SMTs, giving the appearance of entire margins.

Halo of G-genotype colonies

Figures 1E, 1F, and 4A show haloes surrounding some colonies of strain M23 during the active growth stage (5–10 days). Observation of these haloes required bacterial growth at the highly active stage and an optimal focus. In fact, our first observation of haloes came from a computer-image analysis. No similar haloes have been found associated with A-genotype colonies. The chemical nature and biologic function of the haloes remain unknown. Our observations showed that SMTs were confined within the range of the halo (Figs. 1E, 1F, and 4A).

Oblique illumination

Oblique illumination is critical for the observation of movement trails and haloes. Oblique illumination has long been known to provide a high-resolution image (Filler and Peuker 2000). This principle has been used with the compound microscope and reported to reach a resolution that lies between light and electron microscopes capable of detecting a single gene on a human chromosome from an in situ hybridization experiment (Landegent et al. 1985). Stereoscopic observation of bacterial colonies using oblique illumination was first performed by Henry (1933) to differentiate colonies of *Brucella*. Later applications of oblique illumination include bacterial identification (Finkelstein and Punyashthiti 1967) and assessment of the expression of virulent genes (Finkelstein et al. 1992). The use of oblique illumination allowed us to observe *X. fastidiosa* movement trails at a micrometre resolution with an 80× stereoscope. Two other important factors are the use of GelRite as solid support, resulting in highly transparent medium plates, and the high-resolution digital-image analysis.

Conclusion

Xylella fastidiosa is capable of active surface motility, as evidenced by CMTs and SMTs. CMTs were consistent with ALSD A-genotype strains, and SMTs were consistent with ALSD G-genotype strains. Trail formation is common in gliding bacteria, such as *Myxococcus xanthus* (Burchard 1982), which possess twitching motility. Li et al. (2003) demonstrated that the interaction of type IV pili with extracellular polysaccharides on cell and slime-trail surfaces can trigger pilus retraction, resulting in bacterial motility and slime-trailing behaviors. It will be of interest to study whether and how CMT and SMT motilities are related to twitching motility (Meng et al. 2005) and to identify the biologic role of surface motility in *X. fastidiosa*.

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