Supporting Text

Surface Roughness Analysis. The roughness (R) is the standard deviation of the height values (h) away from the mean height (h_0) of a given scan line over the cell surface (Fig. 4). In a 100-nm^2 scan area of the cell surface, the average rms height deviation **[1]** from the mean was calculated for each line in the scan area (Fig. 4) by using the formula

$$R_{rms} = \sqrt{\frac{\sum_i (h_i - h_0)^2}{n}} . \qquad \textbf{[1]}$$

On the scale of 100 nm^2, any artifacts caused by the curvature of the cell body could be ruled out because the cell body was linear. To offset the cell-to-cell variation, a total of 30 100-nm^2 areas on at least 5-10 cells of each cell type were measured (an average of three to six measurements on each cell) and the average R_{rms} was calculated for each strain. Tips from the same batch were used in all experiments to avoid small variations in tip geometry. Roughness analysis was performed on 30 100-nm^2 areas on each cell type presented in this study. A 400-nm^2 area reveals the rough nature of the cell wall of the wild-type cells. (Scale bar, 100 nm.) A typical cross section from which the roughness is measured is shown to the right of the image in Fig. 4 and corresponds to the height profile along the white line. The mean height (h_0) is shown (black dashed line) and the deviation away from h_0 is defined as h_i. (Scale bar corresponds to the height profile.) Using Eq. **4**, we can calculate an average R_{rms} for each cell type by using high-resolution scans of the cell-wall surface: 4.30 ± 1.09 nm, wild type; 2.54 ± 0.77 nm, *dif*; 3.43 ± 0.91 nm, *pilA*$^-$; 7.16 ± 2.74 nm, *stk*; and 4.05 ± 1.42 nm, LPS O antigen.

Fluid Imaging of *M. xanthus* Cells. Wild-type *M. xanthus* cells were immobilized on a glass coverslip, submerged in fluid (see *Materials and Methods*), and probed with AFM. In contrast to imaging in air, the scanning of AFM tip in fluid caused slight movements of cell bodies, which compromised the image quality (Fig. 5). However, these cells were stable enough to be isolated and used for the determination of the local cell-wall elasticity or "stiffness" (Young's modulus, E) by measuring force curves on the cell bodies.

Determination of Young's Modulus. Young's modulus, E, was calculated from force-displacement curves, which were recorded as "approach" and "retraction" curves. During the approach curve, the deflection [$d(z)$] of the AFM cantilever was recorded as a function of the displacement (z) of the piezoelectric crystal, which moves the cantilever toward the sample. The deflection remained at zero as long as the AFM cantilever was off the surface, and increased monotonically after the AFM tip contacted the surface. This increase was linear on a stiff sample but of lower slope and nonlinear on softer samples. The applied force (F) on the cantilever when it approached the cell was determined by multiplying its spring constant (k) by the deflection [$F = k\, d(z)$]. The retraction curve was recorded once a set maximum applied force was achieved, and the cantilever was withdrawn from the surface.

To calculate E, we used a modified Hertz model (1, 2). E can be determined from the equation

$$F = \frac{2}{\pi}\tan(\alpha)\frac{E}{1-\nu^2}\delta^2 . \qquad \textbf{[2]}$$

This model describes how the applied force (F) from the cantilever is related to the tip indentation (d) in a thin elastic sample. In Eq. **2** α is the half opening angle of the AFM tip (36°) and ν is the Poisson ratio of the cell (generally taken to be 0.5). For a stiff sample, the cantilever displacement [$d(z)$] will be equal to the piezoelectric crystal displacement (z), but on a soft sample the cantilever deflection will be decreased by the elastic indentation (δ), as shown in the equation

$$d(z) = z - \delta . \qquad \textbf{[3]}$$

To determine the indentation depth, the contact point (z_0) at which the tip initially contacts the cell must be determined. Because microbial cell surfaces are relatively stiff, we define z_0 as the point at which the cantilever deflection first begins to increase

repulsively from its initial off-contact zero position. At z_0, the change in slope of the force curve, confirmed by examining the first and second derivatives of the force-curve data, allows us to determine the indentation depth (δ) as

$$\delta = (z - z_0) - d(z) . \qquad \textbf{[4]}$$

Using Eq. **4**, we then converted the force-distance curves into force-indentation curves, where E was quantitatively determined by fitting the curves with Eq. **2** (Fig. 6).

1. Touhami, A., Nysten, B. & Dufrene, Y. F. (2003) *Langmuir* **19,** 4539-4543.

2. Rotsch, C., Jacobson, K. & Radmacher, M. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 921-926.