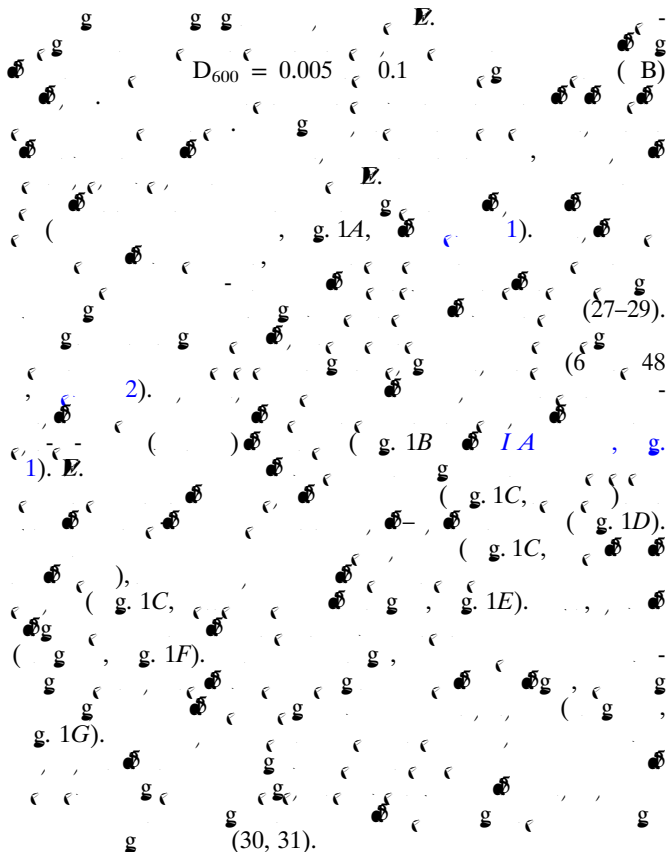
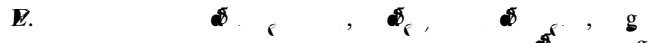
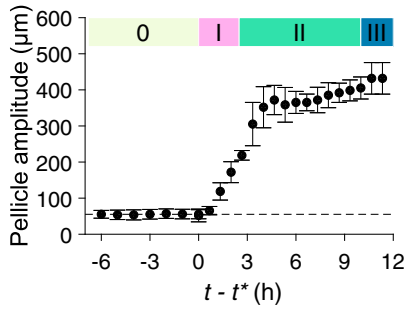


Hierarchical Morphogenic Transitions and Fractal-Order Wrinkling Direct the Standard Mode of *V. cholerae* Pellicle Formation at a Fluid-Fluid Interface

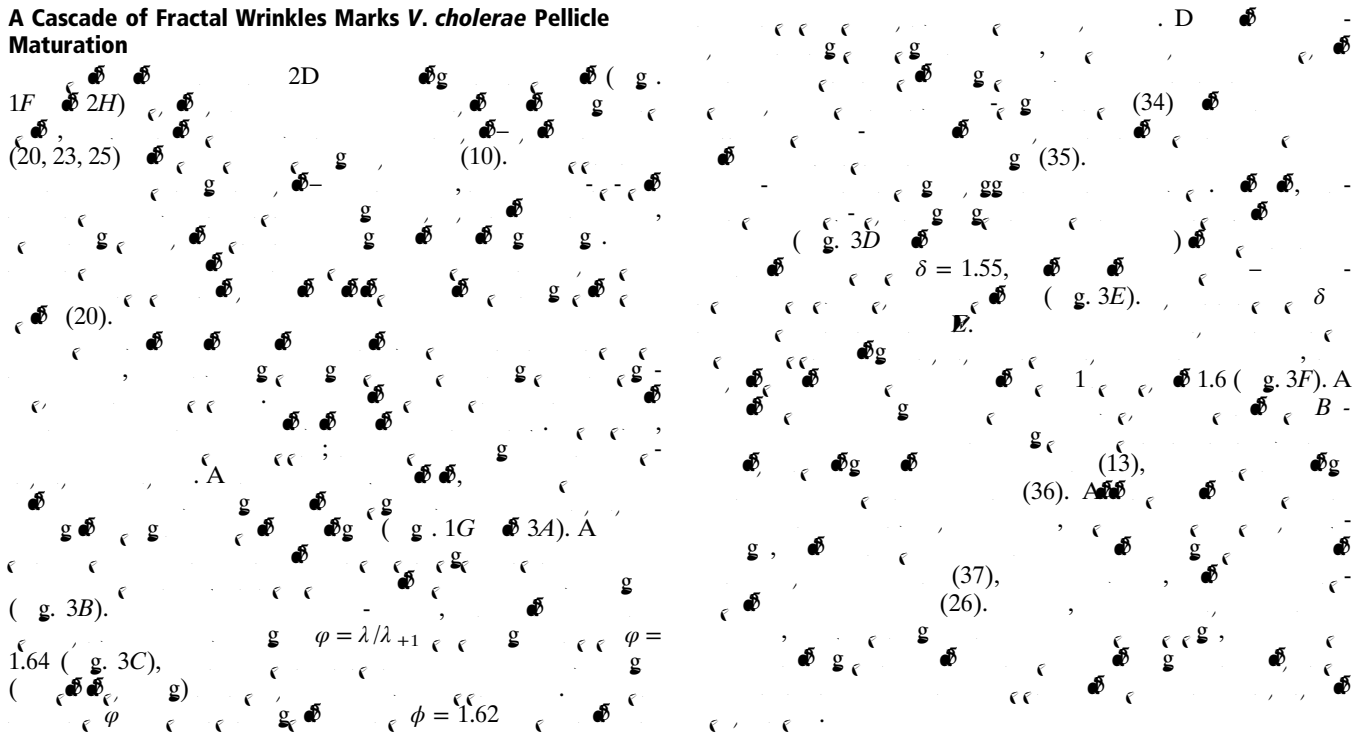


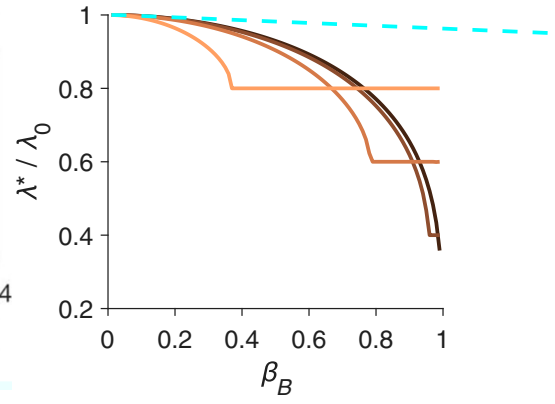
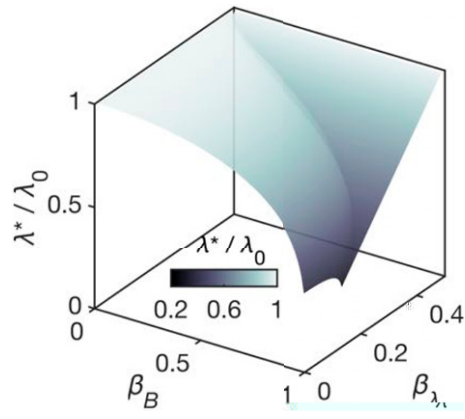
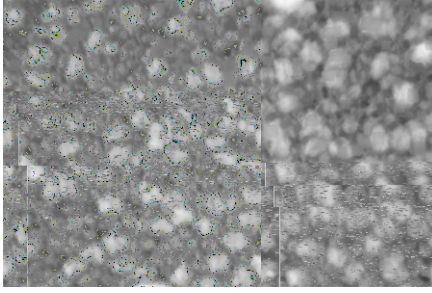
Pellicle Morphogenesis Begins with Microcolony Growth and the Onset of Primary Wrinkling Instabilities





A Cascade of Fractal Wrinkles Marks *V. cholerae* Pellicle Maturation





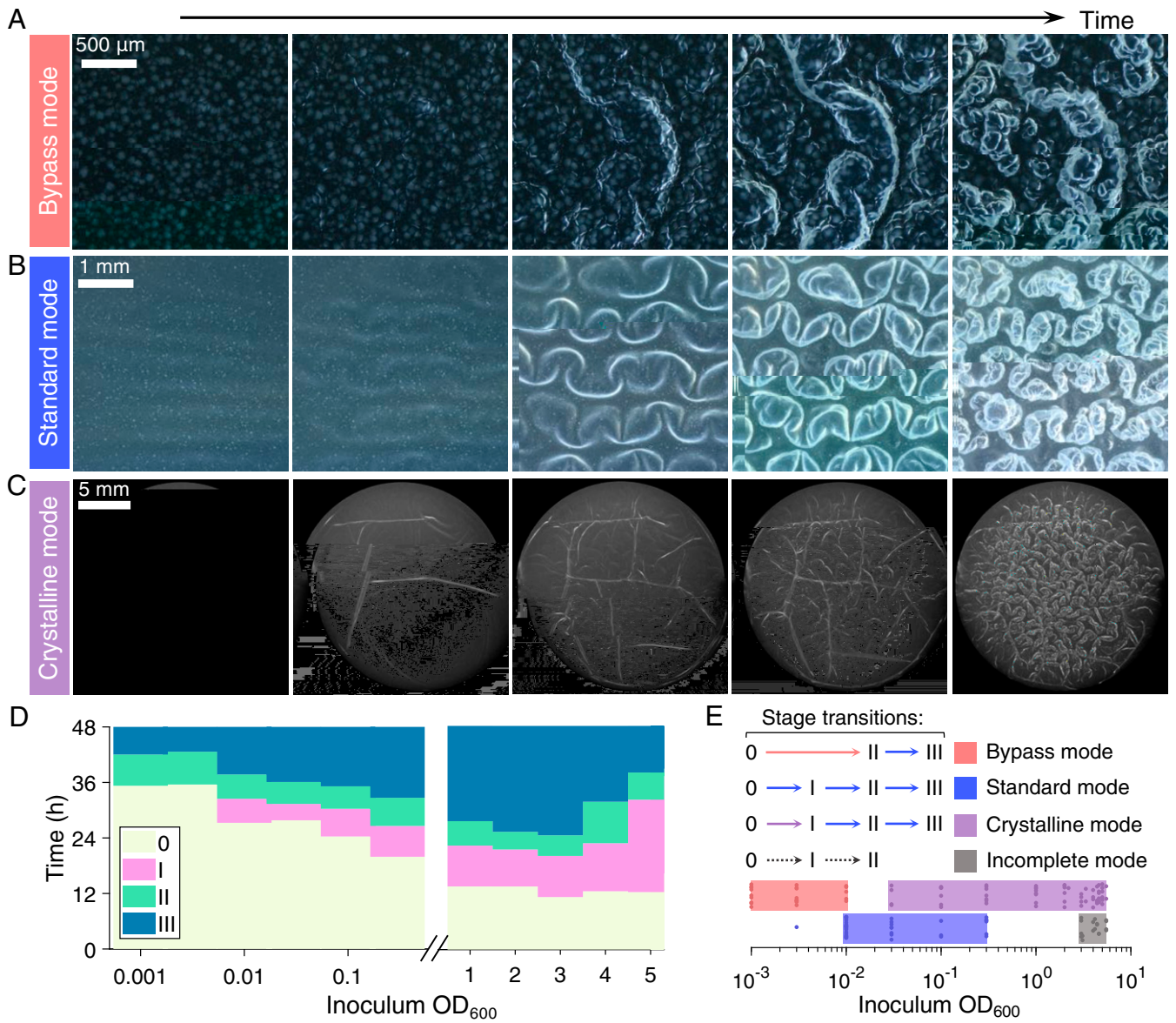
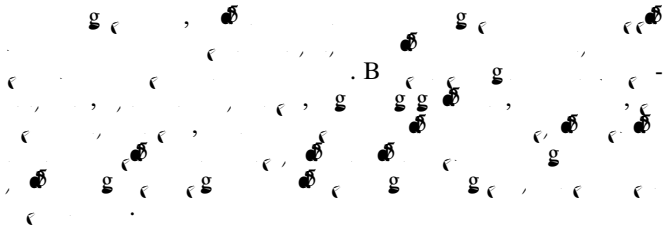


Fig. 5. *V. cholerae*





Materials and Methods

Strains and Growth Medium. *V. cholerae* strains used in this study include the wild-type *V. cholerae* O1 (Inaba) strain 569B, the *vpvC* mutant, and the *Δvc1807::P_{TAC} mNeonGreen Spec* strain. The *Δvc1807::P_{TAC} mKO Spec* strain was also used. All strains were grown in *V. cholerae* growth medium (VPVC).

Pellicle Development. *V. cholerae* strains were grown in VPVC medium in 96-well plates. For pellicle development, cells were inoculated into 200 μL of VPVC medium in a 250 mL flask. The cultures were grown at 37°C with shaking (150 rpm) until they reached an optical density of approximately 0.6. The cultures were then allowed to settle and form a pellicle at the air-liquid interface. The pellicles were harvested and analyzed for surface morphology.

Stereo Microscopy Imaging. Pellicles were imaged using a stereo microscope. The images were captured at two different focal planes to create a 3D effect. The images were then processed to enhance the surface texture and color.

Image Processing. The images were processed using ImageJ software. The images were first converted to grayscale. Then, the background was removed using a thresholding operation. The resulting binary images were used to calculate the fractal dimensions of the pellicle surfaces.

Fractal Dimensions of Pellicle Surfaces. The fractal dimensions of the pellicle surfaces were calculated using the box-counting method. The images were divided into a grid of boxes of size s . The number of boxes containing at least one pixel of the pellicle surface was counted. The fractal dimension D was calculated using the equation $D = \frac{\log(N(s))}{\log(1/s)}$.

Mathematical Modeling of the Primary Wavelength for a Heterogeneous Pellicle. The primary wavelength of the pellicle surface was modeled using a Fourier transform. The images were first converted to grayscale. Then, the Fourier transform was applied to the images. The primary wavelength was determined by the position of the peak in the power spectrum.

$F = \begin{bmatrix} 1 & +\epsilon \\ & \end{bmatrix}$

$\Psi(F) = \frac{\epsilon}{\kappa} (J - \dots) + \frac{Gv}{\kappa^2} (J)$

$G = G(X)$