Using Flow Cytometry to Find Rare Cell Shape Changes in Helicobacter Pylori

November 18, 2013

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Helicobacter pylori is the bacterial agent responsible for most gastric ulcers and stomach cancers. The helical shape of *H. pylori* is critical for the bacteria to penetrate the thick mucus layer of the stomach lining and thus escape the acidic environment of the stomach lumen. Multiple genes are involved in the creation and maintenance of *H. pylori*'s complex shape. Currently, identifying these genes requires laborious microscopic screening of mutant libraries to identify bacterial shape variants. In a report published in *Molecular Microbiology*, Drs. Laura K. Sycuro and Nina R. Salama (Division of Human Biology) employ flow cytometry to substantially enrich cell shape variants and identify new genes involved in the formation of *H. pylori*'s unique shape.

H. pylori cell shape is maintained by a structure between the inner and outer membrane of the bacterium called the peptidoglycan layer, which is composed of alternating glycan chains cross-linked by short peptides. A variety of genes have been found to play a role in determining cell shape, including endopeptidases that hydrolyse peptide cross-links within the peptidoglycan and carboxypeptidases that trim uncross-linked peptides in this layer (Sycuro, *et al*., 2010; 2012). The diversity of identified genes responsible for *H. pylori* cell shape suggests that it is a complex process and that many more shape-determining genes are yet to be discovered. To accelerate this process of discovery, the researchers turned to flow cytometry.

During flow cytometry a stream of droplets, each containing a single cell, pass through a laser beam; light scattered in the forward direction is correlated with cell shape and size. By sorting for bacteria that had different light scattering properties than the wild-type cells, the authors were able to enrich their mutant library for cell shape variants by at least 10-fold, and in many cases by more than 100-fold. After visual screening of the sorted population, 43 clones retained stable rod-shaped morphologies and were selected for further analysis. Most of these clones contained mutations in known cell shape determinant genes; however, the team identified one rod-shaped mutant *H. pylori* carrying a disruption in a novel gene that they named *cell shape determinant 6* (*csd6*).

By analyzing the peptidoglycan isolated from Δ*csd6* mutant *H. pylori*, Sycuro, et al. determined that Csd6 is a carboxypeptidase that cleaves the terminal peptide from a tetrapeptide to generate

tripeptide chains in the peptidoglycan layer. Furthermore, Csd6 is the only source of these tripeptide chains which are then further cleaved by another carboxypeptidase involved in cell shape, Csd4, to generate dipeptides in the peptidoglycan layer. Asymmetrical crosslinking is important for the helical shape of the bacterium, and Csd6 derived tripeptides, but not Csd4 derived dipeptides, can be crosslinked. Therefore, the authors suggest that asymmetrical localization of Csd4 and/or Csd6 along the axis of the bacterium may contribute to the distinctive shape of *H. pylori*.

Sycuro, *et al*. have convincingly demonstrated that flow cytometry is a valuable tool to rapidly and efficiently enrich for cell shape mutants. In addition to the dramatic straight rod morphology changes due to *csd6*, the authors also identified novel mutants with more subtle changes affecting the helical curve of the bacterium, providing further evidence that *H. pylori* cell shape is regulated by a diverse panel of bacterial genes. Additionally, the researchers identified bacteria with transient changes in cell shape, which may suggest kinetic or stochastic regulation of the helical shape. This study opens the door for the rapid identification of cell shape determinants and exciting new studies investigating the regulation of cell shape in heterogeneous populations.

Sycuro LK, Rule CS, Petersen TW, Wyckoff TJ, Sessler T, Nagarkar DB, Khalid F, Pincus Z, Biboy J, Vollmer W, Salama NR. 2013. Flow cytometry-based enrichment for cell shape mutants identifies multiple genes that influence Helicobacter pylori morphology. *Mol Microbiol* 90(4):869-883.

See also: Sycuro LK, Wyckoff TJ, Biboy J, Born P, Pincus Z, Vollmer W, Salama NR. 2012. Multiple peptidoglycan modification networks modulate Helicobacter pylori's cell shape, motility, and colonization potential. *PLoS Pathog* 8(3):e1002603.

See also: Sycuro LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W, Salama NR. 2010. Peptidoglycan crosslinking relaxation promotes Helicobacter pylori's helical shape and stomach colonization. *Cell* 141(5):822-33.

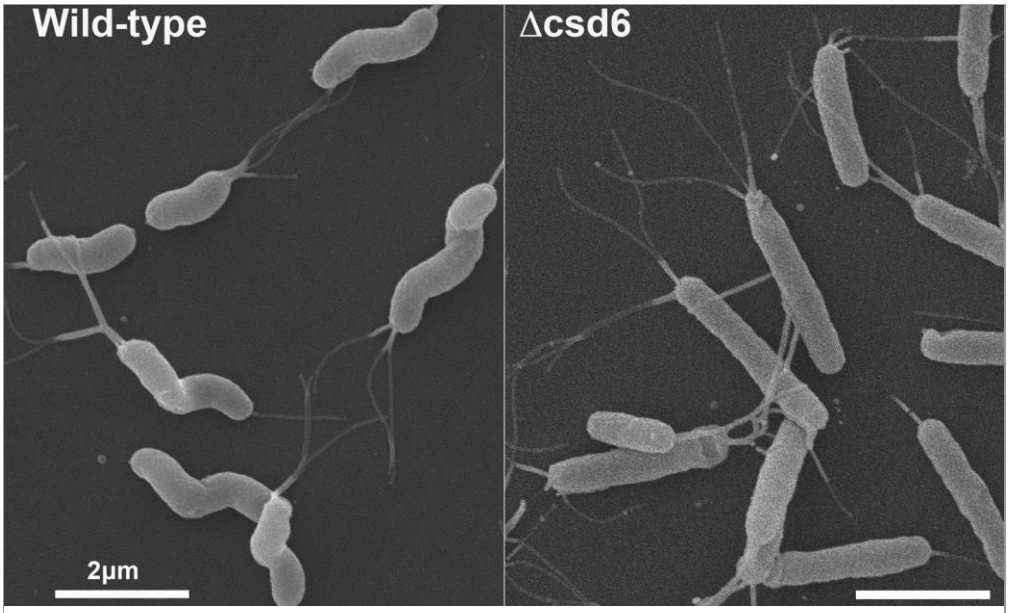

Image courtesy Dr. Nina Salama

Electron micrograph of wild-type shaped (left) or Δcsd6 straight-rod shaped (right) Helicobacter pylori.