Scientists develop new technique for analyzing the epigenetics of bacteria

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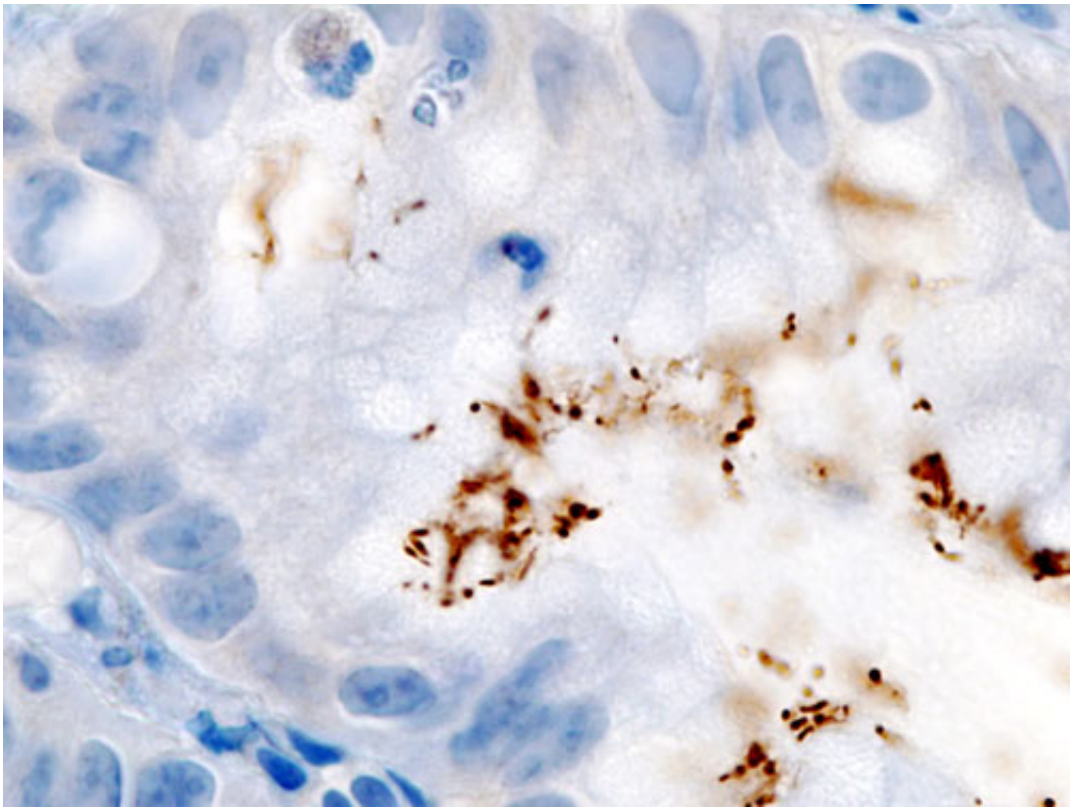

Histopathology of Helicobacter pylori infection in a gastric foveolar pit demonstrated in endoscopic gastric biopsy. Credit: Wikipedia.

Scientists from the Icahn School of Medicine at Mount Sinai have developed a new technique to more precisely analyze bacterial populations, to reveal epigenetic mechanisms that can drive virulence. The new methods hold the promise of a potent new tool to offset the

growing challenge of antibiotic resistance by bacterial pathogens. The research was published today in the journal *Nature Communications*, and conducted in collaboration with New York University Langone Medical Center and Brigham and Women's Hospital of Harvard Medical School.

The information content of the genetic code in DNA is not limited to the primary nucleotide sequence of A's, G's, C's and T's. Individual DNA bases can be chemically modified, with significant functional consequences. In the bacterial kingdom, the most prevalent base modifications are in the form of DNA methylations, specifically to adenine and cytosine residuals. Beyond their participation in host defense, increasing evidence suggests that these modifications also play important roles in the regulation of gene expression, virulence and antibiotic resistance.

The research team employed the PacBio RS II system from Pacific Biosciences, which can collect data on base modifications simultaneously as it collects DNA sequence data. PacBio's single molecule, real-time sequencing enables the detection of N6-methyladenine and 4-methylcytosine, two major types of DNA modifications comprising the bacterial methylome. However, existing methods for studying bacterial methylomes rely on a population-level consensus that lack the single-cell resolution required to observe epigenetic heterogeneity.

'We created a technique for the detection and phasing of DNA methylation at the single molecule level. We found that a typical clonal bacterial population that would otherwise be considered homogeneous using conventional techniques has epigenetically distinct subpopulations with different gene expression patterns' said Gang Fang, Ph.D., assistant professor of genetics and genomics at the Icahn School of Medicine at Mount Sinai and senior author of the study. 'Given that phenotypic heterogeneity within a bacterial population can increase its advantage of

survival under stress conditions such as antibiotic treatment, this new technique is quite promising for future treatment of bacterial pathogens, as it enables de novo detection and characterization of epigenetic heterogeneity in a bacterial population.'

The researchers studied seven bacterial strains, demonstrating the new technique reveals distinct types of epigenetic heterogeneity. For Helicobacter pylori, a pathogenic bacterium that colonizes over 40 percent of the world population and is associated with gastric cancer, the team discovered that epigenetic heterogeneity can quickly emerge as a single cell divides, and different subpopulations with distinct methylation patterns have distinct gene expressions patterns. This may have contributed to the increasing rate of antibiotic resistance of Helicobacter pylori.

'The application of this new technique will enable a more comprehensive characterization of the functions of DNA methylation and their impact on bacterial physiology. Resolving nucleotide modifications at the single molecule, single nucleotide level, especially when integrated with other single molecule- or single cell-level data, such as RNA and protein expression, will help resolve regulatory relationships that govern higher order phenotypes such as drug resistance' said Eric Schadt, Ph.D., founding director of the Icahn Institute and professor of genomics at the Icahn School of Medicine at Mount Sinai. 'The approach we developed can also be used to analyze DNA viruses and human mitochondrial DNA, both of which present significant epigenetic heterogeneity.'

More information: John Beaulaurier, Xue-Song Zhang, Shijia Zhu, Robert Sebra, Chaggai Rosenbluh, Gintaras Deikus, Nan Shen, Diana Munera, Matthew K. Waldor, Andrew Chess, Martin J. Blaser, Eric E. Schadt, and Gang Fang. 'Single molecule-level detection and long read-based phasing of epigenetic variations in bacterial methylomes.' *Nature Communications*. DOI: 10.1038/ncomms8438

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