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Latest News

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Imaging

Viewing Microbial Metabolism

Mass spec paints a picture of nitrogen fixation by individual bacteria in animal cells

Celia Arnaud

Video

Courtesy of the National Resource for Imaging Mass Spectroscopy/Claude Lechene/Harvard Medical School and Brigham & Women's Hospital

This video highlights the journey of nitrogen from the atmosphere into the shipworm via bacterial nitrogen fixation. It zooms from the whole organism to the gill region, first showing a region containing many bacteria and elevated levels of the 15N tracer isotope. It then zooms into a region that has no bacteria but still has elevated 15N levels, indicating that the nitrogen is being used by the shipworm in its own metabolism.

Launch Video

* Macromedia Flash Player 8 is required to view video.

WITH A NEW MASS SPECTROMETRIC imaging method, researchers now can get a close-up, quantitative look at nitrogen fixation by individual bacteria inside animal cells (Science 2007, 317, 1563). Nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia, a form usable in biosynthesis.

The method opens a new way to study the roles of microbes in their natural environments and is already being
applied to other symbiotic systems.

Developed by Claude P. Lechene of Brigham & Women's Hospital and Harvard Medical School and coworkers, multi-isotope imaging mass spectrometry (MIMS) combines nanometer-scale resolution secondary ion mass spectrometry (nanoSIMS) with stable-isotope labeling and quantitative image analysis. In nanoSIMS, a tightly focused beam of cesium ions bombards a surface, continuously ablating the surface and creating secondary ions from the sample that can be measured by mass analysis.

Lechene and his colleagues demonstrate the power of MIMS by measuring nitrogen fixation by individual bacteria located in the gills of an animal called the shipworm (Lyrodus pedicellatus). This marine bivalve dines on wood, which is nitrogen-poor, and so must find other sources of the nitrogen it needs for biosynthesis. It gets that nitrogen from symbiotic nitrogen-fixing bacteria. Only now has that nitrogen fixation been shown directly at the level of individual bacteria.

The researchers feed the worms nitrogen gas enriched in the stable isotope $^{15}$N, turn their cesium beam on the gill region, and then measure the ratio of $^{14}$N to $^{15}$N in secondary cyanide ions ($^{12}$C$^{14}$N$^{-}$ and $^{12}$C$^{15}$N$^{-}$). The incorporation of $^{15}$N can be determined by comparing the measured nitrogen isotope ratio with the natural abundance ratio.

The Harvard team uses a color-coded method to represent the enrichment of $^{15}$N in the tissue. The same ultrathin section can be observed first with transmission electron microscopy (TEM) and then quantitatively imaged with MIMS.

"The overlay of the SIMS image with the TEM image is spectacular and shows how the combination of techniques is so important for nanoscale chemical analysis," says Nicholas Winograd, a chemistry professor at Pennsylvania State University who also develops SIMS imaging methods.

The images show that the nitrogen-fixing bacteria are located in a region of the shipworm gill known as the gland of Deshayes. The $^{15}$N levels were also elevated, though not as much, in nearby bacteria-free gill regions, suggesting that the shipworm is using the fixed nitrogen.

"The images of subcellular structures are of unprecedented quality" for a mass spectral imaging method, Winograd says. "It opens the technique to all sorts of single-cell studies."

Steven Boxer, a chemistry professor at Stanford University, also uses nanoSIMS imaging to analyze the composition of domains in synthetic lipid membranes. "Lechene's work applies the method to a much more complex system," Boxer says. "I think this is a beautiful example of the application of this method to a complex biological problem, where other methods fail, and it demonstrates how useful this can be for biologists."