

recognize spikes in such activities, providing “early warnings” of potential conflicts. Unlike past methods, these methods do not require previous knowledge of the groups being investigated.

Past behavioral models [e.g., to forecast turmoil in Indonesia (6)] were painstakingly built by hand. Building behavioral models in real-time from such data is a challenge that is only now being addressed by software development.

Systems such as the Cultural Reasoning Architecture (CARA) (4) can be used to study the Janjaweed in Sudan (a militia of Arab descent engaged in the systematic use of mass rape and violent attacks against Muslims of non-Arab descent in the Darfur region). Data may include parameters that indicate increases or decreases in these actions. In another example, the probability of suicide attacks by the Lebanese Shiite group Hezbollah when they are not engaged in rocket attacks and car bombings depends upon whether Hezbollah was using education and propaganda as a major part of their strategy. When they were, the probability of suicide attacks was around 47%, but when they were not, the probability shot up to 80%. This is one example of a rule automatically discovered by the CARA architecture (4) with the

“Minorities at Risk” data set (7). The number of possible determining conditions is enormous, and a human analyst could easily miss an interesting hypothesis. Moreover, because programs like T-REX provide a flood of data (45,000 pages per day), sophisticated algorithms are needed. Classification algorithms (8) to identify conditions that neatly separate desirable situations from undesirable ones (e.g., violent actions versus more acceptable forms of negotiation) offer an excellent starting point, although substantial scaling to huge data sets is required.

We can use these methods to model terror groups, political parties, U.S. allies, companies, or regulatory bodies. The final step is to forecast how members of the modeled group may act once a set of determining conditions has been found. Even if we study just 1000 actions, there are  $2^{1000}$  possible sets of actions that a group might take at just the next time point. This corresponds to about  $10^{300}$  possible sets of actions. Current systems such as the stochastic modeling agents in the CARA (4) architecture can estimate the  $k$  most probable sets of action the opponent might take in a few minutes when  $10^{27}$  sets of actions are involved. The ability to access real-time information on these topics, to rapidly analyze the possible actions that interested parties might

engage in, and to determine how best (e.g., with methods of game theory) to respond, will provide a key tactical advantage to organizations that are entering foreign cultures with goals as diverse as stopping terrorism or improving corporate profits.

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## MICROBIOLOGY

# Sizing Up the Uncultivated Majority

Marcel M. M. Kuypers

Coupling the identity of microbes with their activity in the environment remains an important gap in our ability to explore microbial ecology. The development of techniques to quantify the metabolic activity of single microbial cells has been especially challenging, mostly due to their small size. Microbiologists are therefore excited about a new high-resolution imaging method called multi-isotope imaging mass spectrometry (MIMS) or nanoSIMS, which can help decipher what individual microbes are “doing” in the environment. On page 1563 of this issue (1), Lechene and colleagues apply MIMS to identify a symbiotic relationship between a nitrogen-fixing bacterium and an animal host. The technique is poised to reveal the metabolic diversity of the planet’s microorganisms,

99% of which has eluded cultivation (2).

MIMS can determine the chemical, radioisotopic, and stable-isotopic composition of biological material down to the submicrometer level (3–6). By exposing microbial communities to substrates that have been labeled with stable isotopes, MIMS-based imaging allows visualization of metabolic activity in single cells. Moreover, nutrient uptake rates and fluxes can be quantified.

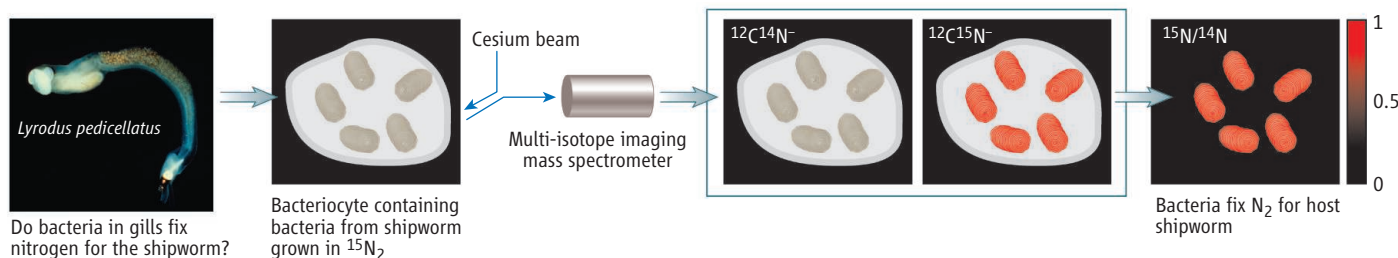
Lechene *et al.* used MIMS to quantify nitrogen ( $N_2$ ) fixation by individual bacteria that inhabit the gills of the shipworm *Lyrodus pedicellatus*. *L. pedicellatus* is a wood-eating marine bivalve with little nitrogen in its diet and must therefore rely on other nitrogen sources (7). Previous studies reported  $N_2$  fixation for intact shipworms, as well as for pure cultures of bacterial symbionts isolated from shipworm gills (7, 8), but neither the site of fixation nor whether the fixed nitrogen is supplied to the host could be determined. Lechene *et al.* grew

A new imaging technique allows the metabolic activity of single microbial cells to be quantified in environmental samples.

shipworms in seawater containing nitrogen gas enriched in the rare stable isotope  $^{15}N$  and used MIMS to measure  $^{15}N$  incorporation in symbionts and shipworm tissue (see the figure). The incorporation of  $^{15}N$  was determined by comparing the quantitative mass images of  $^{12}C^{14}N^-$  and  $^{12}C^{15}N^-$ —produced by bombardment of tissue with a cesium ion beam—to measure the increase in  $^{15}N/^{14}N$  ratios relative to the natural abundance ratio (0.00367). Transmission electron microscopy of the same shipworm gill tissue was used to identify bacteria and host cells. The combined data provide the first direct evidence for in situ  $N_2$  fixation by bacterial symbionts and demonstrate that this nitrogen is used by the shipworm host.

Until the work of Lechene *et al.*, it had not been possible to quantify the incorporation of nitrogen by individual  $N_2$ -fixing microorganisms or to map the fate of fixed nitrogen in the microbial environment. Other methods currently used either do not provide single-cell

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**A new window on microbial activity.** The incorporation of  $^{15}\text{N}$  stable isotope into a mixed population of cells (animal cells and bacteria) is determined by comparing two quantitative mass images ( $^{12}\text{C}^{14}\text{N}^-$  and  $^{12}\text{C}^{15}\text{N}^-$ ) obtained by

multi-isotope imaging mass spectrometry (MIMS). The increase in  $^{15}\text{N}/^{14}\text{N}$  ratios relative to the natural abundance ratio can then be measured to identify the fate of the  $^{15}\text{N}$ .

resolution or, like micro-autoradiography, require that microorganisms be fed radioactive-labeled substrates (9). The uptake of radiolabeled isotopes directly links individual microbial cells to their activity in the environment. However, because this approach requires radioactivity, its use is limited to elements that have a radioisotope with a suitable half-life (>1 day; for example,  $^{14}\text{C}$  and  $^3\text{H}$ ) and excludes the study of other elements such as nitrogen. MIMS, on the other hand, can be used to measure the distribution of any stable isotope as well as any radioisotope with a suitable half-life. Hence, the approach used by Lechene *et al.* holds great promise for studying symbiont-host interactions and microbial activity in the environment.

Combining MIMS with fluorescence in situ hybridization (FISH) is an even more

powerful technique for identifying and characterizing single microbial cells. FISH uses fluorescent-labeled probes that are specific to the organism of interest and that bind to the intracellular 16S ribosomal RNA (2). Replacing fluorescent probes with isotopically labeled (stable or radioactive) or halogenated probes would allow individual cells to be directly identified (by probe hybridization to targets) by MIMS (10). The hybridization procedure is essentially identical to that used for FISH, and the same probes can be applied. By combining this probing technique with isotope labeling of substrate, one can assess the metabolic activity of cells and simultaneously identify their phylogenetic characteristics during a single MIMS scan. This approach links the identity of microbial cells to their in situ activity. MIMS is truly an imaging

breakthrough, whose application is only just beginning to yield information once considered inaccessible.

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## ASTRONOMY

# From Darkness to Light

Volker Bromm

What is the nature of the dark matter that is believed to dominate the structure of the universe at large scales? How did the cosmic dark ages end when the first stars lit up the universe again a few hundred million years after the Big Bang? These questions might be intimately related. On page 1527 of this issue, Gao and Theuns (1) present numerical simulations of cosmological structure formation in the early universe. Their simulations demonstrate how sensitively the formation of the first stars depended on the detailed properties of the still mysterious dark matter. The macrophysics of early star formation might thus hold important lessons for the microphysics of exotic elementary particles.

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According to the standard model (2), star formation in the early universe was very different from the present. Stars today form in giant clouds of molecular gas and dust embedded in the disks of large galaxies like our Milky Way, whereas the first stars emerged inside "minihalos," agglomerates of primordial gas and dark matter with a total mass of a million times that of the Sun.

Another difference arises from the initial absence of elements other than the hydrogen and helium that were synthesized in the Big Bang. Gas clouds today can efficiently cool via radiation emitted by atoms, molecules, or dust grains that contain heavy elements. Because the primordial gas lacked those coolants, it remained comparatively hot. For gravity to overwhelm the higher thermal pressure, the mass of the first stars must have been larger as well. Numerical simulations have led

A supercomputer simulation shows that matter in the early universe might have formed dense filaments before collapsing into the first stars.

most researchers to believe that the first stars were predominantly very massive, typically a few hundred solar masses.

The emergence of the first stars fundamentally changed the early universe at the end of the cosmic dark ages (3). Owing to their high mass, these stars were copious producers of heavy chemical elements that were rapidly dispersed by supernova explosions. They also produced many ultraviolet photons that were energetic enough to ionize hydrogen, the most abundant element in the universe. Thus began the extended process of what cosmologists call "reionization" (see the figure), which transformed the universe from a completely cold and dark neutral state into the fully ionized medium of today. Observations of the polarization in the cosmic microwave background (CMB), due to the scattering of CMB photons off free electrons, place constraints

## Sizing Up the Uncultivated Majority

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