

Mapping the molecules made by a lichen's resident microbes

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An international team of researchers has spatially mapped molecules produced by an intact, complex microbial community for the first time. Using a tiny slice of lichen, the team used imaging mass spectrometry to track and plot metabolites made by both bacterial and fungal lichen members. Their approach, published this week in *mSystems*, an open-access journal of the American Society for Microbiology, shows how researchers can tease apart the chemistry that shapes and maintains a complex, three-dimensional microbial community.

"Until now, there had not been a single microbial community studied from the chemical standpoint—particularly how they are organized with respect to the [molecules](#) being produced," says Pieter Dorrestein, chemistry professor in the University of California, San Diego school of pharmacy and senior author on the study.

The team, which included researchers from the US, Canada, Germany, and Russia, chose the *Peltigera hymenina* [lichen](#), which lives in humid environments on soils in forests and roadsides as a test-bed community. Using a postage stamp sized sample of the lichen collected in British Columbia, Canada, the team first analyzed the relative abundance of all known genes present and found that the lichen was made up of about 81% bacteria (including cyanobacteria) 0.001% archaea, and about 19% eukaryotes (including fungi). More than 75,000 of those genes, or 13% overall, were found to be enzymes involved in producing secondary [metabolites](#).

These metabolites "are really responsible for driving and shaping the microbial community," Dorrestein says. The next steps were to determine which microbial member of the community produces which molecules and where within the lichen.

To do that, the team took scrapings from 110 spots on the lichen sample, extracted the metabolites, and analyzed them by chromatography and mass spectrometry, which gives a 'chemical barcode' for each molecule present. At the same time, the researchers extracted metabolites from lab-grown cultures of 70 microbes isolated from the lichen and performed the same analysis.

Comparing those two sets of barcodes to a global metabolite database allowed the team to identify the producer of about 40% of the total molecules and determine which of the cultured microbes produced certain metabolites. This also gave the team a rough blueprint of which molecules were produced by bacteria, cyanobacteria, or fungi. For example, the metabolites PF1140 and asperphenamate, have antimicrobial and anticancer activities, respectively, and were produced by different fungal isolates in the lab.

Next, the group generated a spatial map of where within the lichen some of these identified molecules resided. Taking a tiny cross-section of the lichen sample, the team used imaging mass spectrometry (IMS) to chart where various molecules appeared. The IMS technology allowed the team to build a color-coded picture of that cross-sectional piece of lichen, showing where each type of molecule is found within its layers. For example, the PF1140 molecule was concentrated in the bottommost layer of the lichen, a portion not exposed to the sun. And not surprisingly, the chlorophyll molecules produced by the lichen's cyanobacteria are concentrated along the topmost, sun-facing layer.

"Now that we have that spatial information, we can build a hypothesis

about what might be PF1140's function," says Dorrestein. Perhaps it is involved in protecting the lichen from soil-dwelling pathogens, he says. Based on the detected chemicals and their locations within the three-dimensional structure, the team concludes that the lichen's fungi are providing defense, structural support, and storing moisture, while the community's cyanobacteria are generating carbon and energy sources.

Studies like this will pave the way for teasing apart the chemical makeup of the complex [microbial communities](#) all around us, says Dorrestein. "If we understand how one or two of these communities are built, we can ask if those principles hold true for other communities, such as those living in our own bodies, on the roots for plants, associated with catheter infections or floating in the oceans."

Provided by American Society for Microbiology

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