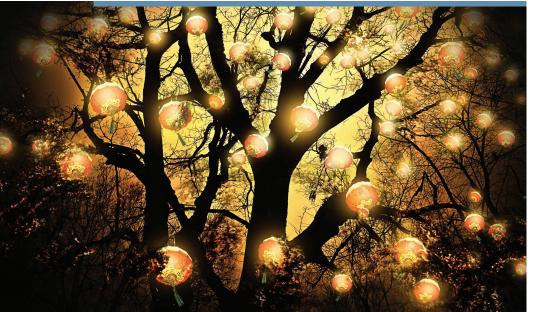
THE PRIMER



autumn 2013 volume 10 issue 3



Single cells shed light on microbial "dark matter"

While reflecting on the rapid increase in microbial genome sequencing and analysis over the past two decades, U.S. Department of Energy Joint Genome Institute (DOE JGI) Prokaryote Program head Nikos Kyrpides observed that, "The remarkable number of microbesalready estimated to be several orders of magnitude greater than the number of stars in the universeurgently calls for a transition from random, anecdotal, and small scale surveys towards a systematic and comprehensive exploration of our planet."

The vast number of unknown and uncultured microbes found in, on and around the planet that are crucial to regulating and maintaining life is referred to as biological "dark matter." In an international collaboration led by DOE JGI researchers, single-cell genomics was used to shed light on more than 200 previously unknown microbial genomes. These findings first appeared online July 14, 2013 in the journal *Nature*. DOE JGI director Eddy Rubin compared the work to "the 21st Century equivalent of Lewis and Clark's expedition to open the American West. This is a powerful example of how the DOE JGI pioneers discovery, in that we can take a high throughput approach to isolating and characterizing single genomes from complex environmental samples of millions of cells, to provide a profound leap of understanding the microbial evolution on our planet. This is really the next great frontier."

This microbial dark matter campaign targeted uncultivated microbial cells from nine diverse habitats: Sakinaw Lake in British Columbia; the Etoliko Lagoon of western Greece; a sludge reactor in Mexico; the Gulf of Maine: off the north coast of Oahu. Hawaii, the Tropical Gyre in the south Atlantic; the East Pacific Rise; the Homestake Mine in South Dakota; and the Great Boiling Spring in Nevada. From these samples, the team laser-sorted 9,000 cells, from which they were able to reassemble and identify 201 distinct genomes, which then could be aligned to 28 major previously uncharted branches of the tree of life. continued on page 3

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Armored Alga is first to have its pan-genome sequenced

MASSIE SANTOS BALLON

When NASA satellites capture images of milky algal blooms in the oceans, the cause is often tiny single-celled organisms with chalky exoskeletons (called coccolithopores) known as *Emiliania huxleyi.* The intricate "armor" lends an air of delicacy to the alga, leading some researchers to call them "flowers of the ocean." Make no mistake, though, the exoskeletons are so strong that they comprise the towering white cliffs of Dover marking the point at which England is closest to continental Europe.

In some marine ecosystems, though the formative process for the alga's "armor" releases carbon dioxide, *Ehux* can trap as much as 20 percent of its organic carbon, derived from CO_2 . *Ehux* can both capture and release CO_2 and thus is potentially of critical importance in understanding marine contributions to climate processes.

"Carbon dioxide is fixed during photosynthesis and calcification," said Betsy Read, a professor of biological sciences at California State University, San Marcos who led a decade-long project and a large international consortium of 75 researchers from a dozen nations in exploring the *continued on page 2*





Armored Alga

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Ehux genome. "It is also released during the process of calcification, but we do not know how this release balances with the amount of CO_2 that is buried when *Ehux* sinks to the bottom of the ocean. This is an important yet unresolved question."

Originally estimated to be about 30 million bases in size, the *Ehux* genome ended up being closer to 141 million bases. "Because of the size and inherent complexities, the genome become known as 'The Beast' and without the epic persistence and unwavering commitment from the DOE JGI, the project would not have been completed," said Read. The *Ehux* genome was compared with sequences from other algal isolates and the results first reported in the June 12, 2013 edition of *Nature*.

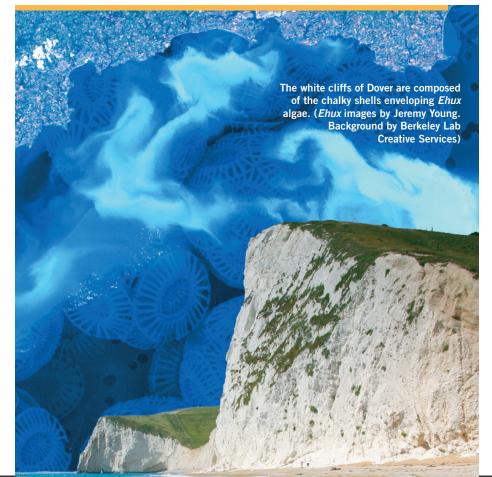
Part of the third most abundant group of phytoplankton, behind the diatoms and dinoflagellates, the *Ehux* strain sequenced by the DOE JGI was isolated from the South Pacific and is the first reference genome for coccolithophores. Ehux and its brethren are the basis of most ocean food chains. Phytoplankton biomass exceeds that of all marine animals combined. Activities of *Ehux* and some other phytoplankton such as diatoms influence climate processes, such as lowering ocean temperatures by reflecting sunlight and through carbon metabolism.

With the advent of next generation sequencing technologies the team was able to conduct a comparison of 13 *Ehux* strains revealing the first ever algal 'pan-genome." *Ehux* doesn't exist as a clearly defined "species" with a uniform genome, but as a more diffuse community of genomes—a "pan-genome"—with different individuals possessing a shared "core" of genes, but supplemented by different gene sets thought to be useful in dealing with the particular challenges of its local environment. "Ehux thrives in a broad range of physiochemical conditions in the ocean," said Igor Grigoriev, the paper's senior author, whose team from the DOE JGI led the genome annotation and analysis. "It's a complex genome, with lots of genes and repeats, the first reference for haptophytes and fills another gap in the Eukaryotic Tree of Life. It is amazing that while you need a microscope in order to see this elegantly sculptured microbe, you can see from outer space the light reflected from large areas of ocean during *Ehux* blooms."

The team found variability in the *Ehux* genome that helps explain the alga's ability to thrive in oceans from the equator to the subarctic and cause algal blooms in the spring and summer that can cover several hundred thousand square kilometers.

"The high diversity within this species indicates that a single strain is unlikely to be typical—or representative of all strains," the team noted. "Future sequencing of phytoplankton isolates will reveal whether this discovery is a unique or more common feature in microalgae. Together, the physiological capacity and genomic plasticity of *E. huxleyi* make it a powerful model for studying speciation and adaptations to global climate change."

Aside from using the DNA to better understand the alga's role in the planet's carbon and sulfur cycles, Read and her colleagues see the availability of the Ehux genome sequence as an important first step to unlocking the molecular mechanisms that govern the nucleation, growth, and nanoscale patterning of the calcium carbonate shells. "We have some clues," she said, "but what makes this more difficult is that proteins involved in calcification are not conserved across biomineralizing species, those that can grow into composite materials. What we desperately need in order to identify the genes is a genetic transformation system. Several labs—including my own—are aggressively working on this." **:**





Single-cell genomics

Microbial dark matter

continued from page 1

"Microbes occupy every conceivable environmental niche from the extreme depths of the oceans to the driest of deserts," said Tanja Woyke, DOE JGI Microbial Program Head and senior author on the *Nature* publication. "However, our knowledge about their habits and potential benefits has been hindered by the fact that the vast majority of these have not yet been cultivated in the laboratory. So we have only recently become aware of their roles in various ecosystems through cultivation-independent methods, such as metagenomics and single-cell genomics."

The team's findings fell into three main areas. The first was the discovery of unexpected metabolic features. They observed certain traits in Archaea that previously only were seen in Bacteria and vice-versa. The second contribution arising from the work was the correct binning of data of some 340 million DNA fragments from other habitats to the proper lineage. This correction provides insights into how organisms function in the context of a particular ecosystem as well as a much improved and more accurate understanding of the associations of newly discovered genes with resident life forms.

The third finding was the resolution of relationships within and between microbial phyla-the taxonomic ranking between domain and class which led the team to propose two new superphyla, which are highly stable associations between phyla. The 201 genomes provided solid reference points, anchors for phylogeny-the lineage history of organisms as they change over time. "It's a bit like looking at a family tree to figure out who your sisters and brothers are," Woyke said. She and her colleagues are pursuing a more accurate characterization of these relationships so that they can better predict metabolic properties and other useful traits that can be continued on page 8



Spotlight: Chris Rinke

Chris Rinke, first author of the *Nature* study on microbial dark matter, notes that the current representation of microbial genomes in the databases is skewed. "More than three-quarters of all sequenced genomes fall into three taxonomic groups or phyla but there are over 60 phyla we know of." He joined the DOE JGI Microbial Genome Program as a computational biology postdoctoral fellow in 2010 after completing his graduate studies at the University of Vienna in Austria and a postdoctoral fellowship at Washington State University-Pullman studying hydrothermal sea vents in the Pacific Ocean. In September, Rinke and his wife Alena Pribyl (shown above) started a year-long ocean expedition on their sailboat, the Green Panther (track their progress at http://www.greenpanther.org). From California, they will sail south to Mexico as part of a shakedown cruise before trying to cross the Pacific Ocean to French Polynesia with the long-term goal of finally ending up in New Zealand or Australia. (*Image courtesy of Chris Rinke*)

Communicating by cartoon

Summer 2013 Public Affairs Intern Emma Schiappacasse (shown in the forefront with left to right—fellow intern Charles Uccifferi and mentors Massie Ballon and David Gilbert) is a college animation major and created a short cartoon about the use of single-cell genomics



to fill in gaps of knowledge about bacteria and archaea in the tree of life. Her video, with a voiceover from DOE JGI's Janey Lee, a co-author on the *Nature* paper, is a complement to the recent study and is available on the DOE JGI YouTube channel at http://bit.ly/DOE JGI-MDM13cartoon. (Image by Roy Kaltschmidt, Berkeley Lab)

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Yellowstone communities

Yellowstone Hot Springs: A Hotbed of Microbial Life

CHARLES UCCIFERRI

Life on Earth finds a way to survive almost anywhere there is a source of energy to support it. In Yellowstone National Park (YNP), this phenomenon is in the extreme by microorganisms that thrive in harsh, acidic hot springs found unsuitable for the rest of Earth's inhabitants.

A team of researchers including DOE JGI scientists set out to investigate how these habitats support microbial life, and to correlate the microbial inhabitants with geochemical variables across different springs in YNP. From the Department of Energy's perspective, the microbes at Yellowstone are of interest to biofuels researchers because they are thermophilic, meaning they thrive in hightemperature environments. Some of these thermophilic bacteria have been found to contain enzymes that break down biomass into sugars that can be deployed in the industrial-scale production of biofuels.

"The idea was to get a comprehensive overview of microbial communities in Yellowstone National Park," said DOE JGI Metagenome Program head Susannah Tringe, an author on a series of papers on Yellowstone microbial communities that appeared in *Frontiers in Microbial Physiology and Metabolism* between May 15, 2013 and June 3, 2013. "Many springs have been studied by independent lab groups, but there had been no coordinated attempt to compare them."

William Inskeep, a professor at Montana State University and longtime DOE JGI collaborator who has dedicated a great portion of his life's research to studying YNP, initiated the project to get in-depth looks at the three separate types of communities



collected from 20 separate sites within the park. Through the DOE JGI's Community Sequencing Program (CSP), Inskeep and his collaborators were able to complete a large-scale project in which he and a team of scientists provided samples of phototrophic (light energy dependent), chemotrophic (chemical energy dependent), and streamer-like (biofilm-forming) community DNA for metagenome sequencing.

The working theory was that certain ecological and geochemical factors contribute to the niche specialization of microbes in YNP. The fruits of the team's labors are now becoming available to the public, allowing insights into microbial community structure and function in geothermal habitats of YNP.

"These high-temperature systems provide model environments in terms of dissecting community structure," Inskeep said. For this reason, each of the three community types was the focus of a separate paper, with their own respective findings, in addition to an overview paper summarizing the complete project.

One of the studies focused on different types of phototrophic

microbial mats that either produce oxygen (oxygenic) or not (anoxygenic). Through shotgun sequencing of several mat types, researchers were not only able to classify many of the organisms present, but also link them to processes like fermentation, carbon fixation, and photosynthesis. These processes help determine why life is sustainable in these microbial mats. The interactions that occur among members of the community are also critical to the function and survival of these organisms. The second type of community studied was the streamerlike microbial mats found in fast-flowing channels within YNP. These communities varied in metabolic and geological traits, with research pointing out that streamer communities are found across a broad pH range and under high and low levels of oxygen. However, because of the way these communities are distributed, the genes associated with energy production show that these microbes have adapted to special niches within YNP.

The third type of community analyzed during the project was Archaea-dominated microbial communities within a pH range of 2.5 to 6.4.

Yellowstone communities

Through Sanger sequencing, researchers were able to determine that Archaea were dominant populations in all environments sampled. Several biological processes like electron transport and energy conservation differed among sample sites, and were consistent with the ecological and geochemical traits of each community. Results obtained from archaeal-dominated communities vielded results consistent with the project's initial hypothesis that ecological and geochemical characteristics (e.g., pH, oxygen versus sulfide) allow these communities to thrive. The project demonstrated that many local ecological factors including temperature, pH, and the sampled depth influence the types of organisms observed.

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Once DNA was isolated from all the sites, researchers were able to analyze the metagenomes to identify the types of microorganisms present. However, many of the organisms whose genomes were sampled have not been cultivated or sequenced. In fact, each data set contained genome sequences from organisms that could not be classified past the level of "Bacteria." However, the cumulative results of the three studies produced a wide array of information in terms of reconstructing the microbial structure of these environments.

"We were able to find at least ten to fifteen major populations with good assembly," Inskeep said. "This project was the first major attempt to look at this many separate communities in this environment." Characterizing the community compositions at these sites is critical for the next steps of linking composition to activities and potentially following them over time.

It is likely that these organisms would not be able to survive independently in these environments, noted the researchers, but the fact that there are not nearly as many distinct populations of organisms present as there are in soil or in water makes it easier for scientists to differentiate between the behaviors of these organisms in their natural habitat, as opposed to a study conducted in a laboratory. By studying these organisms in their natural habitats, researchers can gain insight as to how certain archaea and bacteria thrive through interdependence.

"Research is still ongoing," Tringe



said. "There has been a lot of work with gene expression that can be related to genome data, so we can look at relative gene expression in these communities."

This study was aimed primarily at analyzing how microbes function in the context of a relatively simple community. Looking ahead, most of the work will focus on these extreme microbes to gain knowledge of what they can do based on the study of their genomes. Now that researchers have accomplished a comprehensive study of microbial communities in a narrowly defined environment, they are more confident that it can be done in more complex communities like lakes, oceans, and even soils and permafrost.

As for the near future, Inskeep knows that there is still much work to be done in YNP. He realized in the beginning of the genomic era that there was a clear scientific need to sequence thermophilic communities; if not just for the advancement of biofuels, then for the sheer knowledge of how these communities operate. His projects with hot springs provided an excellent base for continued research within the park, but his work continues in identifying new microbial populations found in YNP.

"This was a great foundational study, but we're just scratching the surface," he said. "We didn't have enough coverage to reveal the whole community structure, so our work continues."

Charles Ucciferri was a DOE JGI Summer 2013 Public Affairs intern. He is studying journalism at San Diego State University.

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DOE JGI Highlights





Streamlining a common survival strategy in marine microbes

Though several thousand microbial species have been sequenced thus far, millions of species remain unknown, in part because they cannot be cultured in the laboratory. One of the challenges in cultivating microbes is that over time, some microbes have pared down their genetic codes to those genes necessary to survive in specific nutrient-poor environments, a process known as genome streamlining.

A marine microbial study published in the July 9, 2013 edition of the Proceedings of the National Academy of Sciences suggests that as finicky as lab-cultured microbes are known to be, free-living microbes are even more so. A team including researchers from the DOE JGI and the Bigelow Laboratory for Ocean Sciences in Maine isolated single microbial cells from samples collected from the ocean photic zones, where photosynthesis takes place, of the Gulf of Maine, Mediterranean Sea, North Pacific and South Atlantic. They then sequenced and assembled draft genomes of 56 single amplified genomes (SAGs). "While other cultivation-independent tools are available, these rarely allow us to assess who in a community does what, a central question in microbial ecology," said Ramunas Stepanauskas, director of the Bigelow Laboratory Single Cell Genomics Center and senior author of this study. "The single-cell sequencing approach allowed us to link an organism's identity and functional gene repertoire for more than fifty key community

members of the ocean photic zone, shedding light on the biology of this ecosystem that covers 70 percent of the planet."

The SAGs were then compared with existing microbial cultures and metagenomic datasets. The team found evidence that unlike the free-living microbes, the cultured microbes thrive in more nutrient-rich environments. "These findings illustrate that bacterial adaptations to nutrient-poor environments are widespread and that dispersal is not limited, but rather water temperature and latitude are key drivers in geographic distribution of certain bacterial groups," said Tanja Woyke, the head of the DOE JGI Microbial Genomics Program and one of the senior authors of the PNAS study. "To put it in other words, bacteria from the sunlight zone can travel large distances, but they then like to settle and thrive in regions of shared temperature and latitude." —MSB



Annotation of an "ennobled" plant flagship genome

Physcomitrella patens has long been the experimental moss of choice for researchers around the world, and was first sequenced by the DOE JGI in 2007. The plant provides insights as to the mechanisms behind plant cell wall synthesis and assembly, mainly due to its accelerated life cycle that allows more comprehensive studies of such processes. In a study published July 23, 2013 in *BMC Genomics*, an international consortium of research-

ers worked with the genes of what the DOE JGI refers to as a "flagship genome," a term meaning that sustained and significant computational and experimental resources are directed to this organism. By using the sequencing information from DOE JGI. the team was able to attribute functions and metabolisms to about 58 percent of all 32,275 predicted genes of the P. patens genome. Researchers expect to use this information to better understand plant biochemical and metabolic processes that will be useful for developing potential bioenergy feedstock crops.

"One of our intriguing findings is that 13 percent of the *Physcomitrella* genes have no clear relatives in any other sequenced organism so far. Analyzing these orphan genes more deeply will reveal the hidden treasures of the moss genome," said the University of Freiburg Chair of Plant Biotechnology Ralf Reski, a senior coordinator on the study, in a statement. The findings from the study were made available at www.cosmoss. org, and further information regarding the moss genome is available through DOE JGI's Phytozome. —*CU*



Breaking down biomass with thermophilic bacteria

The deconstruction of biomass is a pivotal process in the biofuel industry, but the enzymes that possess a significant role in the breakdown of biomass remain relatively unexplored. To this end, DOE researchers at several national labs and user facilities are studying thermophilic bacteria that contain these enzymes.





In a study published July 19, 2013 in PLoS One, a team of scientists including groups from the DOE JGI, the Joint BioEnergy Institute, and the Environmental Molecular Sciences Laboratory (EMSL) researchers set out to further understand the specific proteins involved in biomass deconstruction in a thermophilic microbial community adapted to break down switchgrass, a candidate biofuel feedstock. The DOE JGI team sequenced and assembled metagenomic sequences while the EMSL team identified which predicted proteins are produced by the microbial community.

The group was successful in reconstructing genomes for *Thermus* thermophilus and Rhodothermus *marinus*, two strains that made up a majority of the population samples. In addition, they were able to identify the phyla Paenibacillus and Gemmatimonadetes as important groups involved in biomass deconstruction. The results marked the first time that the functional roles of individual microbial populations within a consortium have been linked with specific enzyme activities. The team was able to identify more than 3,000 separate proteins associated with the breakdown of biomass. but indicated that there are even more unexplored proteins that could serve a purpose in this process. ---CU

research teams with DOE JGI scientists, isolated microbes in soil samples collected from Africa and South America Tunisia and Argentina. Their results were published in the July 2013 issue of *Genome Announcements.* In these studies, researchers assembled draft genome sequences of two members of the *Frankia* genus, known nitrogen-fixing plant symbionts.

Researchers are investigating how ammonia-based compounds from nitrogen fixation are produced. Since some of the strains taken from Tunisia had adapted to dry, arid, high-saline environments, for example, researchers expect that these studies will inform strategies that enhance the productivity of soil to sustain plants in challenging landscapes. This information could prove to be a boon to arid lands where commercial application of natural nitrogen-fixing products could increase biomass feedstock yields for biofuels production. According to the U.S. Department of Agriculture, in 2011, American farmers paid about \$336 per short ton of nitrogen fertilizer, amounting to a total cost of around \$4.3 billion. Supplementing chemical fertilizer by harnessing microbial nitrogen-fixing processes holds promise for diminishing this expense. —*CU*



The importance and function of nitrogen-fixing microbes

In agriculture, fertilizers are often deployed to supplement nitrogen levels in poor soil. To learn more about how microbial nitrogen fixation works, two



Noncoding DNA map provides insight to plant gene regulation

Widely used as a model for plant research, *Arabidopsis thaliana* was the first plant to have its genome completely sequenced. However, the function of its many DNA conserved noncoding sequences (CNSs) remains unclear.

To help answer these questions, the genomes of multiple plants, including A. thaliana and two others sequenced by the DOE JGI, were compared in a study published in *Nature Genetics* on June 30, 2013, Researchers wanted to better understand the factors that account for reduced diversity and gene mutation rate in a species. The team compared three newly sequenced genomes with six previously sequenced genomes including Capsella rubella, whose genome was published June 9, 2013 in Nature Genetics and another brassica species, the salt-tolerant Eutrema salsugineum.

About 200,000 years ago, Capsella rubella began self-fertilizing. A team including DOE JGI researchers sequenced its genome and compared it with C. grandiflora and members of the closely related *Arabidopsis* genus. C. rubella showed a mass decline of the removal of harmful mutations without a naturally occurring alteration in the amount of genes present that can move between chromosomes. From these findings, it is theorized that a dramatic event left C. rubella in a situation where a need for pollinators outweighed the known negatives of inbreeding and caused the C. rubella plant to shift into selfing.

In their comparative study, the scientists identified 90,000 noncoding base pairs, which account for 17 percent of A. thaliana's genome. Their work yielded the first high-resolution, genome-wide map of noncoding regions. The team concluded that the plants retained these sequences because they are vital in the evolution of genome organization. This information, along with ongoing research, will enable scientists to further understand the sequences in plant genomes that control the activation of certain traits in the development of these plants. ---CU and ES

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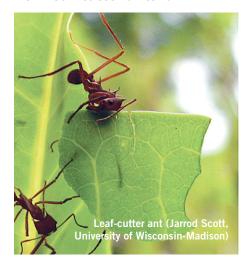
Microbial dark matter -

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expressed by different groups of microbes.

Thus far, cosmologists have only mapped half of one percent of the observable universe and the path ahead in environmental genomics is similarly daunting. "There is still a staggering amount of diversity to explore," Woyke said. "To try to capture 50 percent of just the currently known phylogenetic diversity, we would have to sequence 20,000 more genomes, and these would have to be selected based on being members of underrepresented branches on the tree. And, to be sure, these are only what are known to exist."

DOE JGI Highlights continued from page 7 Fungal genome offers clues on how leaf-cutter ants farm



Leaf-cutter ants are well-known examples of insects that use microbes to gain access to nutrients, farming "gardens" in which bacteria and fungi break down plant mass. In an article featured on the cover of the June 2013 issue of *Applied Environmental Microbiology*, a team including DOE JGI researchers and longtime collaborators at the Great Lakes Bioenergy Research Center reported the first draft genome of a fungus, *Leucoagari*-

The Nature publication "Insights into the phylogeny and coding potential of microbial dark matter" builds upon a DOE JGI pilot project, the Genomic Encyclopedia of Bacteria and Archaea (GEBA: http://www.jgi. doe.gov/programs/GEBA/) and closely articulates with other international efforts such as the Microbial Earth Project which aims to generate a comprehensive genome catalog of all archaeal and bacterial type strains (http://www.microbial-earth.org), and the Earth Microbiome Project (http:// www.earthmicrobiome.org). More information about GEBA-MDM is available at http://genome.jgi.doe.gov/ MDM/. ******

cus gongylophorus, that breaks down plant biomass in the leaf-cutter ants' gardens.

Analysis of the draft genome indicated that the fungus had nearly 150 biomass-degrading enzymes that can break down proteins, xylan, starch and pectin. The finding lends credence to the team's theory that L. gongylopho*rus* plays the primary role in breaking down plant biomass in the fungal gardens. Additionally, a comparison of the soil strata obtained from two different leaf-cutter ant gardens that was done with researchers at the Environmental Molecular Sciences Laboratory revealed that many of the lignocellulases found in the fungal genome were present in the samples, suggesting they played a role in helping to decompose the plant mass collected throughout the 4- to 6-week process.

"The composition of plant biomass in ant gardens is highly variable due to the diversity of plants foraged by the ants and changes in substrate composition throughout the degradation process," the team reported. "Therefore, the ability of *L. gongylophorus* to quickly alter the production and secretion of lignocellulases in response to nutrient availabilities is likely critical for efficient biomass processing." —*MSB*:

The 2014 Department of Energy Joint Genome Institute (DOE JGI) Genomics of Energy & Environment Meeting

March 18-20, 2014 Walnut Creek, CA

Scientists interested in learning about state of the art genome sciences and associated technologies as well as their potential applications to challenges in bioenergy and environmental issues are invited to participate in the 9th Annual DOE Joint Genome Institute Genomics of Energy and Environment Meeting. http://bit.ly/DOE JGI_UM

Notice anything different?

Inspired by the double-helix sculpture in DOE JGI's courtyard, we have "refreshed" our logo. This new look reflects DOE JGI's expanded capabilities as a next generation genomic science center. For the new logo images and style guidance, contact: **degilbert@lbl.gov.**

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