

Scientists decode three bacterial strains common to grapevines and sugarcane

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Scientists at Rochester Institute of Technology have published the whole genome sequence of bacteria associated with Jamaican sugarcane and Riesling grapevines in the September and November issues of the *Journal of Bacteriology*, a publication of the American Society for Microbiology.

André Hudson and Michael Savka, professors in RIT's Thomas H. Gosnell School of Life Sciences within the College of Science, isolated and identified three bacteria belonging to the genus *Enterobacter* from Jamaican sugarcane stalk tissue and *Methylobacterium* and *Novosphingobium* from [grapevines](#).

These genomes—genetic instructions that make up individual organisms—include one of the first to be associated with Jamaican sugarcane, according to Hudson. The scientists deposited the whole genomes at GenBank, a repository maintained by the National Institutes of Health. Hudson and Savka are the first RIT professors to sequence and annotate genomes.

Basic research like their work provides the scientific community with data to apply to the prevention or management of diseases afflicting crops. Sequencing deconstructs an organism's [genetic makeup](#). It deciphers [nucleic acids](#), commonly known as nucleotides—or the "building-block" molecules: DNA ([deoxyribonucleic acid](#)) and RNA ([ribonucleic acid](#)). The specific arrangement of nucleotides, or the genotype, determines the [genetic instructions](#) and denotes an organism's

genus/species in addition to its phenotype, or observable characteristics.

"This study is important because, as the world's population increases, food production and protection will be a big issue," says Hudson, assistant professor of biological sciences at RIT. "There is a lot of emphasis from the USDA and other federal and private agencies that are interested in studying the effects of organisms on [crop production](#) and ultimately food production since 30 or 40 percent of most crops are lost due to pests and pathogens."

Hudson and Savka collaborated with alumnus and lead author on all three manuscripts Han Ming Gan '08 (biotechnology), Sean McGroty '11 (bioinformatics) and Larry Buckley, professor in RIT's Thomas H. Gosnell School of Life Sciences. Ming's collaborators include professors Teong Han Chew from Universiti Teknologi Malaysia and Kok Gan Chan from University of Malaya.

Gan, Savka's former student, is a research scientist at ScienceVision SB in Selangor, Malaysia, with access to the high-through put sequencing and data management technology necessary to sequence and annotate nucleotides. Each bacterium they have studied consists of approximately 5 million to 6 million nucleotides.

The sequencing process breaks an organism down into its basic genetic component and lays the parts side-by-side for scientists to interpret and make useful.

"It's like having a book with no spaces in between the words and you have to find where the words begin and end," says Savka, professor of biological sciences at RIT. "That's what it's like to annotate a genome."

Advancements in sequencing technology helps Gan, Savka and Hudson connect the dots to understand host-bacteria interactions.

"We assembled millions of short DNA sequences into long sequences and made biological sense out of them," Gan says. "Having the near complete genetic information from a bacteria will bring us to a new level of research."

"We can tease out information based on the genome of the organism that live inside the plant," Hudson adds. "The question is, why are these bacteria living in the plants? Are they destroying the plants or are they providing a benefit? Are they providing nutrients that are helping the plant grow, like plant hormones, phosphorous or nitrogen? Is it a mutualistic relationship where the plant and bacteria are both benefiting?"

To answer these questions, the team scoured the [nucleotides](#) for proteins—chains of amino acids that replicate DNA. They found approximately 4,000 to 6,000 proteins in each of the three genomes Gan sequenced.

Experiments Hudson and Savka are designing explore a bacterial communication system known as "[quorum sensing](#)"—or when small molecules act like genetic switches turning on bacterial growth and regulating its pathogenicity. Quorum sensing begins when bacteria grow and produce small molecules until a threshold population is met. With its army in place and ready for orders, the small molecule regulates a coordinated cellular response by the bacteria.

"The bacteria can be coordinated to destroy the plant by producing enzymes that degrade cellulose, lignins or other structures that are important for plant growth and development," Hudson says.

Quorum sensing is Savka's long-term interest and complements Hudson's focus on enhancing nitrogen metabolism in plants.

Sugarcane

Hudson studies the genome of the bacterium *Enterobacter*, an endophyte—or an organism that lives inside a plant—to identify proteins involved in plant growth and development, and which might improve biomass for biofuel production.

Savka and Hudson are looking at *Enterobacter* to determine if the bacterium uses quorum sensing to coordinate activity and whether it is pathogenic to the plant, in addition to its potential role in nitrogen fixation. They are creating genetic mutants of the bacterium to identify genes involved in quorum sensing. Their work is partially supported by a National Science Foundation grant awarded to Hudson.

"The majority of my family works in the sugar industry in Jamaica," Hudson says. "There are a lot of countries where [sugarcane](#) is vital to the economy, like Brazil and India. They do a lot of research, but not so much in Jamaica because it's a third-world country and it doesn't have a lot of the scientific infrastructure. But it has the same problems. I am hoping to find new genes for nitrogen metabolism."

Grapevines

The team's Riesling grapevine research could lead to novel applications for improving disease resistance to organisms that attack the xylem, or vascular system, of grapes.

Sap-feeding insects create wounds and doorways for bacteria to enter a plant's xylem. Pathogenic endophytes weaken a wide variety of economically important crops like grapes, citrus, plum, peach and coffee, among others. Savka began exploring grape endophytes in 2006 while on sabbatical at the New York State Agricultural Experimental

Station, an extension of Cornell University in Geneva, N.Y.

Few studies have characterized bacteria from xylem fluids of grapevines, says Savka. The strain he is looking at produces proteins that exhibit the coordinated communication signaling that might influence the spread of pathogenic bacteria. Savka and undergraduate students in his lab made a genetic mutant of a protein from *Methylobacterium* that doesn't produce quorum sensing signals. Savka and Gan will continue this line of research in future studies.

"Ming has access to the technology to do the global studies," Savka says. "We have formed a collaboration with Ming to determine the whole genome picture, a global picture of what genes are regulated by the small quorum sensing molecules. It is a very powerful tool to have."

The third bacterium the team decoded colonizes a plant tumor disease in grapevines, stone fruits, nut trees, pomes, woody ornamentals and fruit canes. *Novosphingobium* spreads the disease caused by a Nopaline bacterium associated with crown gall tumors. The disease causes uncontrolled tissue growth at the site of the bacterial infection and can significantly decrease crop yields, Savka says. The sequenced strain of bacteria that helps spread the disease was isolated from a tumor affecting a Hungarian Riesling grapevine. The strain was shown to produce the cell-signaling communication that mobilizes molecules.

Provided by Rochester Institute of Technology

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