Michael Schofield inherited two research projects as a student in the lab of David Sherman at the University of Michigan Life Sciences Institute. The first identified two novel marine organism-derived antibiotics that can kill anthrax and drug-resistant staphylococcus bacteria. The second landed her in a kayak in a mangrove forest in the Florida Keys.

There, she collected samples that allowed her and her colleagues to pinpoint the true origins of a cancer drug produced inside of the mangrove tunicate, a sea squirt that grows in bright orange colonies on submerged tree roots. Their findings mark an important step toward greatly simplifying how the drug is made and may prove instructive for developing new drugs from other sea creatures.
Scientists have known for almost 50 years that extracts from the tunicate can kill cancer cells. At the 1969 Food-Drugs from the Sea Conference, researchers funded by the National Cancer Institute and National Institutes of Health presented findings from a broad survey of sea life. Among 132 species of animals and plants sampled, five produced substances that extended the lives of cancer-stricken mice — but the big standout were the tunicates, “whose extract brought about actual cures in two separate trials.”

The active agent was ultimately identified and named ecteinascidin-743, after the tunicate's scientific name: Ecteinascidia turbinata. In the beginning, there were big questions about how sustainably the drug could be obtained — more than a ton of the tiny invertebrates had to be harvested in order to produce a single gram of the medicinal chemical, a 1996 article in New Scientist noted. But by 2000, scientists had figured out how to synthesize larger quantities of ET-743 in the lab, though it requires a laborious, multistep transformation of an antibiotic known as cyanosafracin B.

Today, ET-743, also known as trabectedin, has been approved for use in patients in Europe and is in clinical trials in the U.S. Yet while researchers long suspected that bacteria, rather than the tunicates themselves, actually make the drug, its precise source had remained a mystery — and a roadblock to improving production.

“You can imagine what a huge difference it would make if we could culture the bacteria that makes the drug directly,” says Schofield. “It would also open up opportunities to genetically modify the organism to produce new, related compounds.”

Schofield and her colleagues didn’t know much about the microorganism they were after when they set out.

“We didn’t know if it lived inside the host animal’s cells or on the outside,” says Schofield, who received her Ph.D. in microbiology and immunology this spring from the Program in Biomedical Sciences at the U-M Medical School. “So we tried a lot of different techniques to release the bacteria in the samples — we stuck them in a blender, we ground them up with a mortar and pestle.”

These mixed-up collections of genetic material from the tunicates and all the microorganisms living on and inside of them were sent to the Department of Energy’s Joint Genome Institute in California for DNA sequencing.

“Usually, when we’ve done sequencing in the past, we’re sequencing an isolated bacterium,” Schofield says. “The big challenge here was working with the whole metagenome — not just DNA from our bug, but all sorts of other bugs, and the animal itself.”

To sort through the soup of genetic data, Schofield collaborated with bioinformatics specialist Sunit Jain and associate professor Gregory Dick, Ph.D., from U-M’s Department of Earth and Environmental Sciences. Jain shared first authorship with Schofield when their research was published earlier this year in Environmental Microbiology.

“Symbiotic microbes have long been thought to be the true sources of many of the natural products that have been isolated from invertebrates in the ocean and on the land — but very little is known about them because it’s extremely difficult to identify them and culture them in the lab,” says Sherman, the Hans W. Vahlteich Professor of Medicinal Chemistry in the College of Pharmacy and an LSI faculty member.
Sherman adds, “Currently, many of these compounds can only be harvested in small amounts from host animals, which is unsustainable from an economic and environmental perspective. Our hope is that understanding the genomes of these microorganisms and the chemical reactions that occur inside of them will provide new avenues to economical and sustainable production of the medicinal molecules they make.”

Back in 2011, the Sherman lab sequenced other tunicate zooids — the scientific term for individuals within a colony — and identified a partial gene cluster responsible for producing ET-743. It spanned some 35,000 DNA base pairs, which, written out, would be about the length of a *New Yorker* profile.

“So in the new samples, we were looking for that piece of DNA, and we found it,” says Schofield.

This enabled Schofield and her colleagues to zero in on a particular bacterium, *Candidatus* Endoecteinascidia frumentensis, as the cancer drug’s originator — and ultimately to piece together its entire genome. Not only is the bacterium a new species, it appears to be an entirely new family, she notes.

The bacterium’s genetic code also proved to be far smaller than most. The microbe that produces ET-743 is a mere 631 kilobases in length, 86 percent smaller than *E. coli*, which has a genome containing 4,600 kilobases.

“We were really shocked when we saw the size,” she notes. “We had to go through and triple-check it. This was our first clue that what we were looking at lived inside the cells of its host and was pretty dependent on it.”

Somewhere back in their history, the tunicates and the bacteria were independent creatures, but over time, they evolved a cooperative relationship that allowed the microorganism to shed large swathes of its DNA.

“Our best guess is that the host animal is using the toxicity of the ET-743 produced by the bacterium as a defense mechanism,” says Schofield. “And it’s become essential enough to the tunicate that the bacterium has lost a lot of the genes it would otherwise need to survive on its own.”

This interdependence is precisely what makes culturing ET-743 in the lab so difficult — the bacterium is no longer equipped to live and grow outside of its host.

“But now that we have the genome, we can look and see what genes are missing. We can look at what essential amino acids it was getting from the tunicate and provide them to it in laboratory experiments,” she adds.

And that’s exactly what they’re already doing. The Sherman lab has partnered with Xiaoxia “Nina” Lin, Ph.D., an associate professor of chemical and biomedical engineering at U-M, to try to culture *Candidatus* Endoecteinascidia frumentensis in her laboratory.

“There are some challenges, but I’m optimistic,” Lin says, noting recent advances in scientists’ ability to grow previously “uncultivable” bacteria.

One of the first hurdles was the difficulty of getting the bacteria out of the tunicate cells while still keeping it viable, she says. They’re also working to fine-tune the levels of oxygen and nutrients that will keep the bacteria happy.

“We got a lot of clues from the metagenome,” says Lin, who uses a variety of advanced techniques to investigate microbial systems for applications from biofuels to health.

Meanwhile, Schofield, who shares credit on two pending pharmaceutical patents, is eyeing a career beyond the lab as a medical and science writer. Other students have already inherited the ET-743 work and are carrying it forward.