Genetic technology reveals how poisonous mushrooms cook up toxins

November 12 2007

Heather Hallen spent eight years looking for poison in all the wrong places. Alpha-amanitin is the poison of the death cap mushroom, Amanita phalloides. The Michigan State University plant biology research associate was looking for a big gene that makes a big enzyme that produces alpha-amanitin, since that's how other fungi produce similar compounds. But after years of defeat, she and her team called in the big guns – new technology that sequences DNA about as fast as a death cap mushroom can kill.

The results: The discovery of remarkably small genes that produce the toxin – a unique pathway previously unknown in fungi.

The discovery is reported in today's *Proceedings of the National Academy of Sciences*. It is work that not only solves a mystery of how some mushrooms make the toxin – but also sheds light on the underlying biochemical machinery. It might be possible one day to harness the mushroom genes to make novel chemicals that would be useful as new drugs.

"We think we have a factory that spits out lots of little sequences to make chemicals in Amanita mushrooms," said Jonathan Walton, MSU plant biology professor who leads Hallen's team. "Our work indicates that these mushrooms have evolved a mechanism to make dozens or even hundreds of new, previously unknown chemicals, besides the toxins that we know about."

Of the thousands of species of mushrooms, only about 30 produce alpha-amanitin. Most of them look much like their edible cousins. But poisonous mushrooms are powerful in folklore and in history. In 54 A.D., Emperor Tiberius Claudius was fed a death cap mushroom by his wife Agrippina to put her son Nero on the throne of Rome.

Alpha-amanitin kills people by inhibiting an enzyme necessary for expression of most genes. Without the ability to synthesize new proteins, cells quickly grind to a halt. The intestinal tract and the liver are the hardest hit as they come into first contact with the toxin. By the time symptoms show up, a liver transplant is often the only hope.

Hallen, a mycologist, gathers mushrooms in the Michigan woods and often is called upon to help identify mushroom species for veterinarians, parents of small children and local hospitals – often in a desperate race to beat alpha-amanitin's effects.

Walton's lab works to understand the biochemical pathways by which natural products are
synthesized in fungi. Fungal natural products that benefit human health include penicillin and the immunosuppressant drug cyclosporin. Studying their biosynthesis could lead to the discovery and development of new medicines.

To find the elusive gene for alpha-amanitin, they used what they term "brute force" – a new machine at MSU that can sequence immense quantities of DNA quickly. The 454 LifeSciences pyrosequencer generates 100 Mb DNA sequence in one overnight run - twice the size of a fungal genome. Traditional sequencing methods require months to yield the same quantities. What they found was a gene that encodes the toxin directly – with no need to first synthesize an enzyme that in turn would make the toxin.

“The RNA goes in, and out comes the backbone of the toxin,” Hallen said. After its initial synthesis, the toxin is then modified in several ways by the mushroom to make it exceptionally poisonous.

Walton said the discovery poses some interesting evolutionary questions. For example, why do only some mushrooms produce this toxin" And how did a handful of other, unrelated mushrooms evolve the same trait" Finding the genes points to how the trait could appear in one mushroom, but not how it evolved in mushrooms that aren’t related to Amanita.

Hallen and Walton also see the doors opening to a diagnostic test that could use DNA to determine if a mushroom is toxic or not. Identifying a mushroom by shape and color alone is often impossible if the mushroom has been cooked or partially digested, yet rapid and accurate identification in an emergency room situation is critical.

Source: Michigan State University