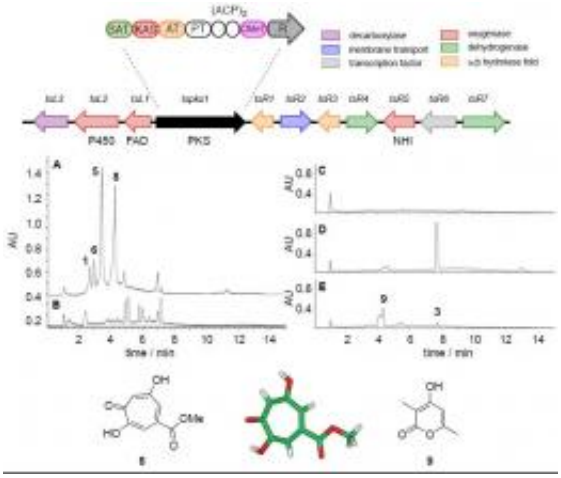
Friendly Fungi: Elucidating the fungal biosynthesis of stipitatic acid

May 18 2012, by Stuart Mason Dambrot



Involvement of tspks1 (tropA) in the biosynthesis of methylorcinaldehyde and tropolones in T. stipitatus. PKS domains: SAT, starter unit acyl transferase; KAS, ketosynthase; AT, acyl transferase; PT, product template; ACP, acyl carrier protein; CMeT, C-methyl transferase; R, acyl CoA thiolester reductase. HPLC analysis of tspks1 KO: (A) UV chromatogram at 260 nm for WT T. stipitatus; (B) UV chromatogram at 260 nm for T. stipitatus tspks1 KO. HPLC analysis of tspks1 expression in A. oryzae: (C) UV chromatogram at 293 nm for untransformed A. oryzae; (D) UV chromatogram at 293 nm for A. oryzae expressing aspks1; (E) UV chromatogram at 293 nm for A. oryzae expressing tspks1. Copyright © PNAS, doi: 10.1073/pnas.1201469109

(Phys.org) -- In a tale worthy of Sherlock Holmes, scientists in the School of Chemistry at the University of Bristol, UK have solved a biochemical mystery that had previously proven elusive for 70 years:

How the fungus *Talaromyces stipitatus* produces stipitatic acid (6), which is a *tropolone*, one of an atypical group of fungal natural products – that is, small molecules produced by genetically encoded pathways – with a seven-carbon ring. (Most natural products, such as cholesterol or phenylalanine, have five or six carbons in rings.) The researchers used a two-part biosynthetic approach – gene deletion and alternate genetic expression – to investigate the molecular pathway in question.

Professor Russell J. Cox, Postgraduate student Jack Davison and other researchers engaged in the study faced several long-standing obstacles to showing that 3-methylorcinaldehyde is the direct product of a fungal nonreducing polyketide synthase (NR-PKS) which most likely appends the methyl group from S-adenosyl methionine during biosynthesis of the tetraketide, and which uses a reductive release mechanism to produce the observed aldehyde. “Gene knockouts have been one of the most useful tools in the toolkit of the biosynthetic chemist,” Cox tells Phys.org, “but knockouts can't answer complex questions like these. We now make extensive use of heterologous expression – that is, moving the gene to a 'clean' host and switching it on.”

The scientists then monitor the host organism for the production of new compounds which we isolate and identify. The chemical structure of the new compound tells them a great deal about the chemistry which must have been used to make it. “In this case,” Cox continues, “we showed that the TropA gene encodes a polyketide synthase which makes 3-methylorcinaldehyde. Expression also allows us to do more complex experiments – for example, by truncating or mutating the gene – and in this way we discovered the reductive release mechanism and the fact that the programmed methylation occurs during chain extension rather than after chain-building and ring formation.”

Tropolone biosynthesis was one of the longest-standing problems in the field of biosynthesis and some of the most distinguished organic

chemists of the last century were fascinated by it, but progress had been very limited. “We realized that combining chemical knowledge with genome sequence data could give us the start we needed,” Cox explains. “We already knew a lot about polyketide biosynthesis in fungi, and this allowed us to narrow down the potential genes involved to just four. We then used chemical knowledge to narrow this further to a single gene cluster.” Proof then came from the knockout and expression studies.

Looking ahead, the team is currently working on systematic methods to express many genes in fungi. “At present this is easy in bacteria, because in bacteria a single promoter can switch on lots of genes in parallel,” notes Cox. “In fungi, however, each gene needs its own promoter, so this has limited progress. In collaboration with our colleagues in the School of Biological Sciences at Bristol we’re developing systems which can express a dozen or so fungal genes in parallel. This will allow the researchers to investigate much more complex systems in the future.

In addition, Cox adds, “I do believe that computational biology and chemistry will eventually provide answers to complex questions like this – but at the moment, while computational methods allow us to formulate good questions, lab work is still needed to find the answers. We’re working in an area with very many unknowns, so it’s difficult for computational methods which rely on current knowledge to act predictively with any accuracy. In fact,” notes Cox, “this is a powerful reason why fundamental discoveries are still so important: they’ll form the basis for future predictions.”

Cox points out that he and his team will also continue to study the TropB-D enzymes in vitro. “Chemical methods of classical enzymology will allow us to determine their precise mechanisms.”

Cox also articulates how comparing the T. *stipitatus* tropolone biosynthetic cluster with other known gene clusters allows clarification

of important steps during the biosynthesis of other fungal compounds, including the xenovulenes, citrinin, sepedonin, sclerotiorin, and asperfuranone. “Fungal genomes generally are much bigger than their bacterial counterparts by roughly 10 times, and they contain many more genes and gene clusters encoding the biosynthesis of complex compounds” he explains. “Barely any of the known fungal gene clusters have been linked to the molecules they must encode. Our work now allows the understanding of a set of genes which encodes the biosynthesis of polyketides followed by oxidative modifications and these occur frequently in fungi. Until now these clusters were mysterious, but now we can – at least partially – begin to understand what they may do. Secondly, knowledge of the gene clusters will allow us to go hunting for new clusters more effectively.” For example, puberulic acid is a potent antimalarial compound, but its gene cluster is unknown – and the team predicts that the cluster should be very similar to the *T. stipitatus* tropolone gene cluster.

In terms of other research and applications that might benefit from their findings, Cox says that understanding biosynthetic pathways is a key strand of the new science of Synthetic Biology. “One can think of the gene clusters and biosynthetic enzymes they encode as building blocks of new biological entities. In the future,” he concludes, “it will be possible to combine the genetic and chemical knowledge of biosynthetic pathways to produce bioactive compounds – such as drugs and agrochemicals – using biology rather than chemistry. This offers huge advantages in terms of sustainability.”

More information: *Genetic, molecular, and biochemical basis of fungal tropolone biosynthesis*, *PNAS* May 15, 2012 vol. 109 no. 20 7642-7647, doi: 10.1073/pnas.1201469109