

Sequencing the Genome of the Cannabis Plant

By Mark Lewis

Canadian researchers led by Jonathan E. Page of the University of Saskatchewan in Saskatoon reported at this year's ICRS meeting that cannabis has evolved an elegant chemical mechanism for the production of olivetolic acid, a precursor molecule in the biosynthesis of cannabinoids.

Biosynthesis is the series of steps by which a complex compound is made inside cells by enzymes acting on (catalyzing) precursor compounds.

The identification of olivetolic acid cyclase (OAC), the enzyme responsible for catalyzing production of olivetolic acid, clarified a major element in the cannabinoid biosynthetic pathway. Discovery of OAC's catalytic activity also uncovered an unexpected evolutionary parallel between cannabis and bacteria.

These findings by Page and colleagues were preceded by their "Draft genome and transcriptome of *Cannabis sativa*," which identified the sequence of some 500 million base pairs in the DNA of a drug-type chemovar (Purple Kush) and a fiber-type (Finola).

The genome analysis was published last year in the open-access journal *Genome Biology* (<http://genomebiology.com/2011/12/10/R102>).

The genome is the sequence of base pairs coiled in a double helix that constitutes an organism's DNA. Genes are sections of DNA that contain "recipes" for making proteins. The nucleus of every cell in the organism contains identical DNA, but not every cell makes use of the same genes to perform its functions. Which genes get turned on — "expressed" in a given cell nucleus — depends on which are chosen to become part of the transcriptome — "transcribed."

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The transcriptome is created by messenger RNA, which lies alongside the DNA and selects certain genes to copy. The selected genes — the transcriptome — is then processed by ribosomes and from its recipes, the specified proteins are assembled.

For the transcriptome study conducted by Page *et al*, living cannabis cells were taken from different parts of the plant during various stages of development. As expected the photosynthetic tissues — leaves — from both drug-type and fiber-type expressed similar genes, especially during their vegetative growth phases.

In early flowering, both drug-type and fiber-type cells exhibited an increase in the expression of RNA transcripts that encode for proteins that are used in the synthesis of cannabinoids. The differences between fiber and drug cannabis emerged later, in flowering, when the process of cannabinoid synthesis ramps up.

Finola, the fiber cultivar, is known to produce CBD acid. As expected, Finola cells produce RNA transcripts with CBDA synthase. By contrast, there was virtually no CBDA synthase being expressed in the cells of the Purple Kush drug cannabis variety.

Although Purple Kush did not produce significant amounts of CBDA pathway transcripts, it did express high concentrations of genes involved in the THCA biosynthetic pathway, as expected from a high-THC drug cannabis cultivar.

The ability of Purple Kush to mass-

Mark Lewis, PhD, is a natural products chemist with Napro Research, a California-based laboratory.

produce THCA is intriguing because, historically, plant domestication tends to involve a reduction in secondary metabolite production. The opposite trend has been observed with drug cannabis. In fact, the up-regulation of the THCA biosynthetic pathway in Purple Kush appears to be the consequence of the longstanding effort to selectively breed higher THCA cannabis (to maximize psychoactive effect).

A Future in Pharming?

So what is the link between cannabis and bacteria? The olivetolic acid cyclase (OAC) gene that was just discovered in the *Cannabis* plant has a close cousin encoded in the genome of the bacteria genus *Streptomyces*. Bacteria and cannabis have evolved similar ways of assembling molecules like olivetolic acid. If similar enough, one can simply isolate the key genes for biosynthesis, mix and match them, and who knows what goodies might emerge? This strategy for drug development — "combinatorial biosynthesis" — is widely used by drug manufacturers today.

Genetically modified *Streptomyces verticillus* is currently being used to produce bleomycin, a peptide-derived natural product used in the treatment of testicular cancer. Using cannabis plants to grow anticancer compounds other than cannabinoids is an unlikely prospect, but companies are already looking at tobacco trichome cells to produce novel drugs. Perhaps someday, the secretory cells in cannabis will be harnessed to produce new medicines within their sophisticated phytochemical factories.

Dr. Page's Perspective

The University of Saskatchewan, where Jonathan Page is adjunct professor of biology, ran a very informative piece about his research on its website. An excerpt follows:

Page explains that the pathway is an unusual one, involving a specialized version of one enzyme, called hexanoyl-CoA synthetase, and another enzyme, called olivetolic acid cyclase (OAC), that has never before been seen in plants.

"What cannabis has done is take a rare fatty acid with a simple, six-carbon chain and use it as a building block to make something chemically complex and pharmacologically active," Page says.

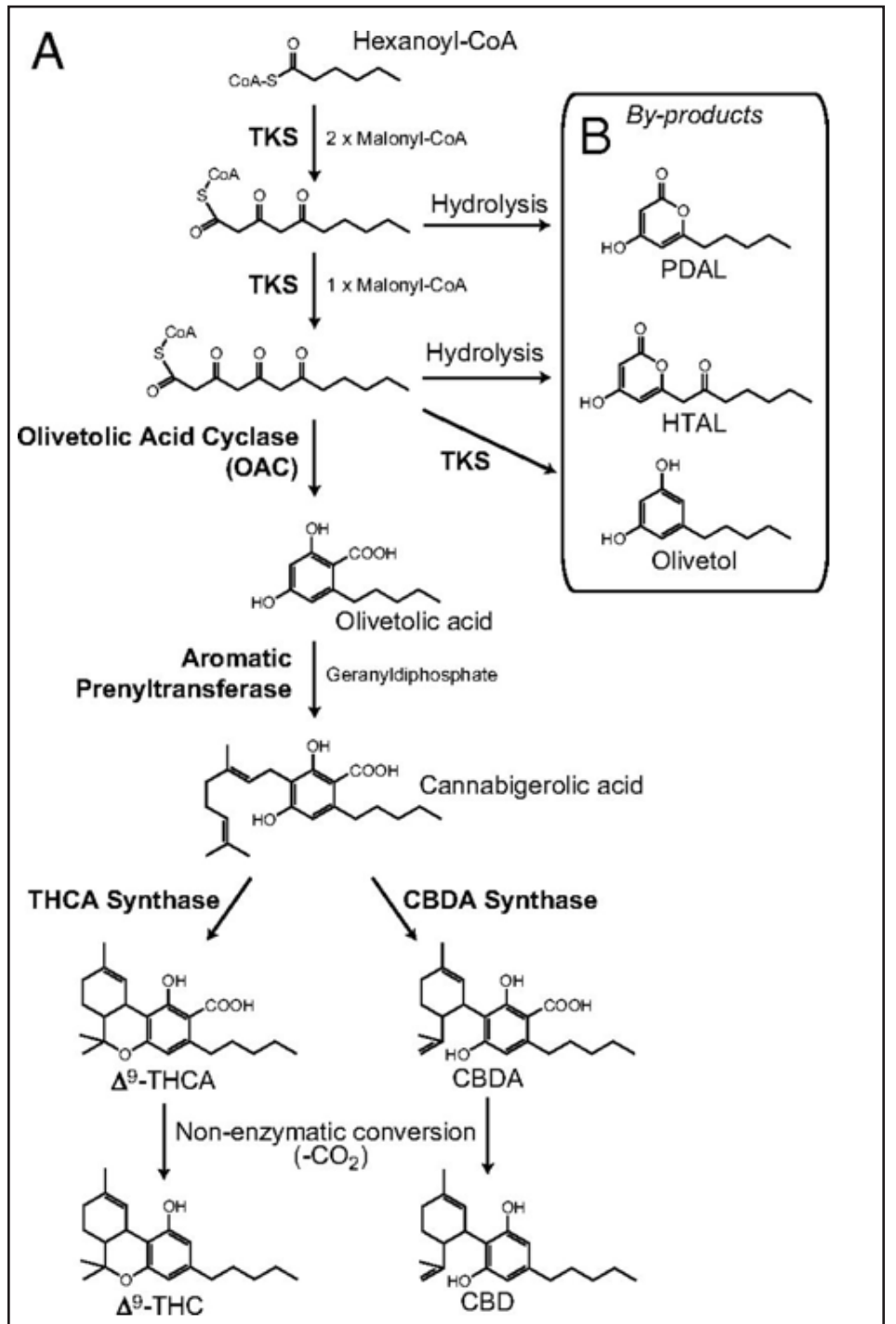
Page led the research with PhD student Steve Gagne, who discovered OAC, and postdoctoral researcher Jake Stout, who discovered hexanoyl-CoA synthetase (reported earlier this year in *The Plant Journal*).

Cannabinoids such as delta-9-tetrahydrocannabinol, or THC, are produced on the flowers of the female plant in tiny hair-like structures called trichomes, the plant's own "chemical factories." The researchers used genomic analysis of isolated trichome cells to produce a catalog of the genes involved in cannabinoid production.

Producing cannabinoids with yeast or other microorganisms could be a valuable alternative to chemical synthesis for the pharmaceutical industry.

Page and his colleagues have already used the new enzymes to coax yeast to produce olivetolic acid, a key metabolic intermediate in the biochemical pathway that leads to cannabinoids.

"Now that we know the pathway, we could develop ways to produce cannabinoids with yeast or other microorganisms, which could be a valuable alternative to chemical synthesis for producing cannabinoids for the pharmaceutical industry," Page says.



PROPOSED BIOSYNTHETIC PATHWAY (A) leading to the major cannabinoids THC acid and CBD acid, which decarboxylate to THC and CBD. Insert (B) shows recombinant TKS enzyme produces PDAL and tetradetide (HTAL and olivetol) by-products in vitro.



TRICHOMES, the globular resin glands on top of stalks extending from a Cannabis flower (reportedly Skunk#1 strain) were photographed in connection with Jonathan Page's research into the genome and transcriptome of cannabis. Cannabinoids and terpenoids secreted by the trichomes are responsible for the medical effects of Cannabis. Photo was made at an electron microscopy facility run by Dr. Klaus Adler of IPK-Gatrsleben, a German institute for plant breeding.

"One of the miraculous things about the cannabinoids is that they're only found in the Cannabis plant. But the precursors are found in other plants! Olivetol is in lichens! What is the lichen using olivetol for? It might be worth looking into," added Dr. J, sounding a bit like Mechoulam himself.

Photo of lichens by Mark Dimmitt

