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Researchers monitor cannabis propagated using plant tissue-culture techniques at Ebbu in Evergreen, Colorado.

BIOTECHNOLOGY

A boosted crop

Genetic engineering could enable cannabinoids of pharmaceutical interest to be produced on an industrial scale.

BY ELIE DOLGIN

Cannabis is the only plant known to produce tetrahydrocannabinol (THC), but it remains an imperfect vessel for producing the chemical on an industrial scale. The psychoactive substance is normally found only in small outgrowths from the plant known as trichomes, which means that its stalk, stems and leaves are wasted biomass.

Genetic engineering could provide more efficient alternatives. Some researchers and biotechnology companies are aspiring to replace cannabis plants with microorganisms that have been genetically enhanced to spit out THC, the non–psychoactive compound cannabidiol (CBD) and myriad other cannabinoids of pharmaceutical interest. Others are aiming to modify chemical synthesis in the cannabis plant by genetically altering its cells to make the desired molecules from shoot to tip, thereby boosting yield.

Either way, the goal is the same: to produce cannabinoids more cheaply, efficiently and reliably than by conventional plant cultivation in greenhouses or farmers' fields. Further benefits of microbial synthesis include the ability to mass-produce rare cannabinoids that are usually present in plants in only trace amounts — or even molecules not found in nature. Transgenic plants can also be engineered for superior resistance to pests and environmental stresses.

Commercial interest in these strategies is picking up. In 2018, for example, Canopy Growth Corporation in Smiths Falls, Canada — the largest legal cannabis company in the world — paid more than US$300 million in cash and shares to acquire Ebbu, a small company in Evergreen, Colorado, that had developed one of the earliest platforms for manipulating the cannabis genome with the gene-editing system CRISPR–Cas9. And in April, Zenabis, a cannabis producer based in Vancouver, Canada, agreed to purchase 36 tonnes of almost-pure, bacterial-made CBD from medical-cannabis company Farmako in Frankfurt, Germany — the first deal of its kind for biosynthetic cannabinoids.

David Kideckel, a cannabis analyst with financial-services company AltaCorp Capital in Toronto, Canada, describes genetic engineering as a "disrupter" that promises to take a centuries-old agricultural practice into the biotechnology era, with the resulting ripples being felt throughout the cannabis sector worldwide. When it comes to producing cannabis extracts, plants could be supplanted by microbes, and a greater range of cannabinoids could become available for use in medical and recreational products.

If that happens, the iconic cannabis leaf would no longer accurately represent where the active ingredients come from. Instead, a stainless steel bioreactor might be more apt.

COOKING UP CANNABINOIDS

Part of the appeal of ditching greenhouses for bioreactors boils down to cost. Currently, 1 kilogram of high-quality CBD extracted from plants sells for a wholesale price of more than $5,000. A deal in 2018 between Ginkgo Bioworks, a synthetic-biology company in Boston, Massachusetts, and Cronos Group, a Toronto-based cannabis producer, outlines a plan to manufacture pure CBD and other cannabinoids for less than $1,000 per kg in yeast.

CASE STUDY

Pot's patent predicament

Under federal law in the United States, the cultivation of cannabis is strictly prohibited. But that hasn't stopped the growth of the country's cannabis industry, which has been operating in a quasi-legal fashion since individual states began to allow the sale of cannabis for medical and recreational use more than 20 years ago. Nor has it stopped the US Patent and Trademark Office from granting intellectual-property licences for cannabis breeding and production.

One such patent sent shockwaves through the industry. Granted in 2015 to a company called Biotech Institute in Westlake Village, California, it covers a range of cannabis varieties with appreciable levels of tetrahydrocannabinol and cannabidiol.

The sweeping nature of the patent's claims concerned many cannabis breeders, who feared that it could stifle innovation and biological diversity in the fledgling cannabis sector. They also worried that artisanal marijuana production, which is driven by consumers' needs and tastes, could be supplanted by an age of corporate cannabis.

Other broad patents have followed, as have legal disputes. In 2018, for example, two Colorado-based firms were embroiled in a lawsuit over whether one company's liquid formulation of hemp-derived cannabidiol infringed on the patent claims of the other. It was the first high-profile patent challenge in the sector. The case is ongoing.

The issue in that lawsuit, and in others, is whether the patent is novel — and therefore worthy of protection — or an obvious development in light of prior art. Because of cannabis's long history of hidden cultivation, breeders have not chronicled their varieties in the public sphere. Consequently, patent examiners had little information on which to base decisions on whether cannabis-related technologies are new and non-obvious. That lack of a paper trail also makes it hard to mount a proper challenge to a patent.

Beth Schechter hoped to change that. As executive director of the non-profit organization Open Cannabis Project (OCP), Schechter and her team built a public record of chemical and genetic profiles of hundreds of existing cannabis varieties that were submitted by members of the community. The goal was to provide evidence to show that some patents are obvious and therefore invalid, she says, and "if nothing else, to at least prevent similar patents like those going forward".

But the project might end up having unintended consequences. Although touted as a way to protect the rights of small farmers, it folded in May after a video emerged of OCP co-founder Mowgli Holmes pitching to investors the idea of an in-house breeding programme at Phylos Bioscience, a cannabis-science company in Portland, Oregon, that he co-founded and now leads as chief executive. For many, it confirmed their fears: that OCP was a front for Phylos to amass cannabis data for financial gain.

According to Holmes, Phylos was only seeking to publish data through the OCP, and "None of the customer data had any value to a plant breeding program." Yet the damage was already done.

"Making data public is good because it enlarges the public domain and it speeds up science," Holmes maintains. But in the emergent cannabis industry, secrecy and intellectual property continue to define battle lines. **E.D.**

Biomanufacturing also offers a level of consistency that is impossible to replicate in plants, which, like most agricultural commodities, are subjected to the weather, pests and other environmental uncertainties. Laboratory-based production is also better for the environment because less energy is needed to run a bioreactor than to power the grow lights and ventilation fans of an indoor cannabis-growing operation. The water pollution and land destruction that is associated with outdoor cannabis cultivation (see page S8) can also be avoided.

Perhaps the biggest advantage of cooking up cannabinoids in fermenters, however, is the ability to brew copious amounts of lesser-known cannabinoids that are usually found only in trace amounts in cannabis plants.

"People are so focused on the big two — THC and CBD — that we're sort of forgetting that there are potentially other really useful compounds in the plant," says Tony Farina, chief scientific officer at synthetic biology company Librede in Carlsbad, California. "That's the direction for which we should really be using this biosynthesis platform."

Cronos has singled out a few molecules of particular interest. These include cannabichromene, a rare cannabinoid that is thought to have anti-inflammatory properties, and cannabigerol (CBG) — a chemical precursor to THC and CBD with the potential to protect cannabis plants from damage-inducing molecules inside cells. High on the company's list is also an appetite-suppressing variant of THC called tetrahydrocannabivarin (THCV). This cannabinoid has medical potential in people who are affected by compulsive overeating disorders, and THCV could appeal to recreational users of cannabis who enjoy the drug's intoxicating effects but would rather avoid its hunger-inducing properties.

"It offers the same euphoric effect as THC, but without the munchies," says Cronos chief executive Mike Gorenstein.

At least 18 companies are racing to produce cannabinoids in yeast, bacteria or algae. Although each industry player has a proprietary approach, all are variations on the basic playbook described earlier this year by synthetic biologist Jay Keasling at the University of California, Berkeley (X. Luo *et al. Nature* **567**, 123–126; 2019).

Keasling and his colleagues introduced a series of genetic changes into the yeast *Saccharomyces cerevisiae*. By tweaking some yeast genes, and inserting others from bacteria and the cannabis plant, the team created an organism capable of carrying out all the chemical reactions that are involved in cannabinoid production. Feeding the yeast a simple sugar generated low amounts of inactive THC or CBD, which can be converted into their active forms by heating.

Because the enzymes in the cannabinoid pathway are "a little sloppy", as Keasling puts it, the team could also introduce fatty acids that the yeast would incorporate into cannabinoids. This spawned variants of THC and CBD that are not found in nature. "We created entirely new molecules that might be better therapeutics," Keasling says.

At the yields reported, however, Keasling's platform is not ready for prime time. Dramatic improvements in both the yeast's efficiency and the fermentation protocol are needed for the biosynthetic approach to be cost-competitive with plant-extracted cannabinoids. Demetrix in Emeryville, California — a company co-founded by Keasling that has secured more than $60 million in funding, making it the best-financed start-up company devoted to lab-based cannabinoid production — is developing the technology further. Demetrix chief executive Jeff Ubersax says that his team has increased the cannabinoid yield by "several orders of magnitude".

But many companies made similar claims to *Nature* that, without verifiable data, cannot be substantiated. Even if they are true, getting something to work in the lab does not guarantee success in a manufacturing plant, says Stephen Payne, chief executive of Maku Technologies, a start-up in Durham, North Carolina. Maku is focusing on making rare, natural cannabinoids in yeast. "Throughout my time in the synthetic-biology industry, I've seen things work on a small scale that have no chance of reaching industrial levels," Payne says.

CATALYSING SUCCESS

Turning yeast into miniature cannabinoid factories poses considerable challenges. Although Keasling's protocol involves 16 genetic modifications, the overall efficiency of the procedure came down to a single bottleneck.

The log-jam involved an enzyme that is needed for CBG production. Researchers

characterized the enzyme, known as a prenyltransferase, around a decade ago in a strain of medical cannabis. Initially, Keasling tried to use that cannabis-derived enzyme in yeast, but it didn't work: the yeast produced no CBG.

After rummaging through gene-expression databases, however, Keasling found an alternative prenyltransferase that was encoded by another variety of cannabis. He introduced this into the yeast and all the pieces fell into place to make CBG and its derivatives.

Some researchers faced the same enzymatic challenge in *S. cerevisiae* and elected to switch to alternative organisms. Bioengineer Oliver Kayser and his colleagues at the Technical University of Dortmund in Germany turned to a species of yeast called *Komagataella phaffii* (B. Zirpel *et al. J. Biotechnol.* **259**, 204–212; 2017).

> ***"Intellectual property will rule the day in this space."***

Others have sworn off yeast completely. Vikramaditya Yadav, a chemical engineer at the University of British Columbia in Vancouver, has moved to working in bacteria instead. He is collaborating with a Vancouver-based company called InMed Pharmaceuticals to produce cannabinoids in *Escherichia coli*.

One advantage of bacteria over other cell-based systems, says Yadav, is that they don't attach sugars to the proteins that they produce in the same way as yeast and other organisms with an enclosed nucleus do. Those sugar adornments can limit the activity of enzymes that are crucial to the cannabinoid pathway — at least in *K. phaffii*, as Kayser's team has shown (B. Zirpel *et al. J. Biotechnol.* **284**, 17–26; 2018) — which leads to lower yields.

Bacteria also naturally secrete the cannabinoids that they produce into the surrounding medium, from which they can be extracted easily. This phenomenon provides speed and cost advantages because it enables continuous manufacturing, whereas organisms that retain their chemical bounty inside cells must be 'cracked' open as part of a batch-production system. Yeast do not typically secrete proteins, but researchers at Librede and elsewhere claim to have engineered this function into the organism.

A further challenge for using either yeast or *E. coli* is the toxicity of cannabinoids. Such molecules evolved in plants as a defence mechanism against insects, microorganisms and other biological threats. This means that the chemicals that researchers desire are often deadly to the organisms that have been engineered to make them.

At Farmako, which announced in July that its biosynthesis research team would be spun off to form a new biotechnology company, scientists therefore turned to *Zymomonas mobilis*, a bacterium used in tequila production. According to molecular biologist and Farmako co-founder Patrick Schmitt, who is expected to lead the spin-out company, this microorganism is immune to cannabinoid toxicity — although it's not clear why. Meanwhile, researchers at Renew Biopharma in San Diego, California, are working in *Chlamydomonas reinhardtii*, a green alga that compartmentalizes its cannabinoid synthesis in chloroplasts. In so doing, the rest of the cell is shielded from the toxic molecules.

As well as the biological advantages, cannabinoid production in an unconventional organism such as an alga makes good business sense because the approach is proprietary, says Michael Mendez, founder and chief executive at Renew Biopharma. "Intellectual property will rule the day in this space," he says. And as Jeremy de Beer, a law professor at the University of Ottawa who has studied cannabis patents, points out: "We're in sort of an intellectual-property gold rush."

Already, the US Patent and Trademark Office has protected Librede's use of yeast to synthesize cannabinoids from sugars. Other patents have followed, including one that was granted to Teewinot Life Sciences in Tampa, Florida, for a bioreactor designed to grow cannabinoid-producing microorganisms. Legal battles might not be far behind (see 'Pot's patent predicament'). "It will not be a surprise at all, as revenues from cannabis sales pick up, that you see similar increases in patent-related enforcement," says Stephen Hash, a patent attorney at Baker Botts in Austin, Texas. "It will go hand in hand."

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Crystals of purified cannabidiol oil.

FIRMLY PLANTED

Rather than trying to force the production of cannabinoids in microorganisms, some companies are sticking with cannabis plants, but using biotechnology tools to give the crop a boost.

Trait Biosciences in Toronto has genetically engineered cannabis to enable it to produce cannabinoids throughout the plant, not just in the trichomes, to increase the yield that each plant provides. The company also added enzymes that made the cannabinoids less toxic and made the usually oily molecules soluble in water.

"That was a side benefit that we soon realized was perhaps as important, if not more important, than the yield boost," says Richard Sayre, Trait's chief science officer. "Now that they're water soluble, we can essentially press the plant just like they do with sugar cane to squeeze the juice out and recover the cannabinoids."

Water solubility also opens up the possibility of creating new kinds of cannabis-infused beverages or edible products. "It's tasteless and odourless, so it can be blended in a variety of applications," Sayre explains.

At Ebbu, director of genetic research Robert Roscow has filed patents that cover methods for manipulating cannabinoid synthesis in plants. He uses CRISPR–Cas9 gene editing to delete certain enzymes in the cannabinoid-synthesis pathway that are involved in THC production. This has enabled him to generate cannabis plants that produce only CBD. And by targeting enzymes that are involved in both THC and CBD synthesis, he has produced plants that secrete only CBG.

Some skilled cannabis growers have created plants rich in minor cannabinoids such as CBG or THCV through selective breeding alone, but that can be a laborious and difficult process. "Modification through genetic engineering is probably the most straightforward way to get a desired phenotype," says Igor Kovalchuk, a plant biotechnologist at the University of Lethbridge, Canada, and co-founder of cannabis-genomics company InPlanta Biotechnology, also in Lethbridge.

Genetic engineering is also a powerful tool for probing the function of cannabis genes — information that can then be fed back into a more conventional breeding programme. But beyond the lab, Kovalchuk says, "I don't believe that genetically engineered cannabis has a future for years to come."

One obstacle remains consumers' skittishness about genetically modified crops, which could carry over to a distrust of microorganism-based biosynthesis. "People like their weed, and they will care if their cannabinoids are coming from a genetically modified yeast or a field-grown plant," says Jordan Zager, co-founder and chief executive of Dewey Scientific, a cannabis biotechnology company in Pullman, Washington.

The technological provenance of cannabinoids might not matter as much to the pharmaceutical sector, where consumers tend to be less averse to genetic engineering. But according to Ethan Russo, director of research and development at the International Cannabis and Cannabinoids Institute in Prague, biochemically derived cannabinoids, even when mixed and matched into therapeutic formulations, will probably never equal the botanical synergy of the hundreds of molecules that are found in cannabis.

The existence of this 'entourage' effect is not universally accepted (S12). But to Russo, "The plant is nature's design for this panoply of chemicals". ■

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