BREWING UP BREAKTHROUGHS

With precision tools, synthetic biology methods offer hope against some of the major global challenges. Simon Lawrence spoke to UC Berkeley's Professor Jay Keasling about altering yeast's genes to create medicines and fuels.

Available at supermarkets for a few bucks and existing since before recorded history, brewer's yeast is too commonplace to excite most people.

Routinely used to make bread and ferment alcoholic beverages, *saccharomyces cerevisiae* has long been a dietary staple, and has been studied extensively as a model organism.

University of California, Berkeley biochemical engineer Professor Jay Keasling is excited about yeast. He has spearheaded high-impact research on the microbe, tweaking its innards so it can digest sugar and excrete what eventually becomes antimalarial treatment. This could potentially save countless lives.

Synthetic biology has reached a point where the DNA of an organism can almost be edited at will.

"Over 15 million people have gotten access to those treatments produced using the engineered yeast," he tells *create*, nominating the work as the most satisfying he has been involved in.

S. cerevisiae and other microbes, through the application of synthetic biology, can be made to produce all kinds of useful things.

They're like "little chemical factories", believes Keasling, and their metabolic pathways able to be altered to serve numerous ends. In a world with an increasing strain being applied to its finite resources, manmade microbial strains could be crucial in solving many of our biggest problems.

One leading synthetic biologist, Harvard's Pamela Silver, has suggested that biology, rather than anything else, is the technology of the 21st century. Does Keasling agree?

"Of course it's the technology of the 21st century! Why not?," he declares, almost sounding insulted by the question. "It's going to have enormous implications."

Synthetic biology

The definition of synthetic biology – a mix of biology, computer science, engineering and other disciplines – is elusive, and depends on who you ask.

"Genetic engineering on steroids" is one attempt, and Keasling has suggested it's simply "engineering biology to do things it wouldn't normally do."

The title goes back to the mid-70s, around the time genetic engineering was emerging, though its principles began to be articulated early this century.

Leaders such as Keasling, George Church and Drew Endy stressed the electronics engineering paradigm as a model. The ability to build circuits from standardised parts and devices, and consistent improvements in technology enabled by this, are cited. The goal was to borrow from the approach of planar process manufacturing.

Early problems addressed by the field have been in areas such as diagnosis, chemical manufacturing, and biofuels.

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Though synthetic biology hasn't led to affordable, renewable fuel for all, the discipline has gotten much more sophisticated.

An iteration – in which new life is literally designed and created – is now only weeks in the making, where it might've formerly taken years. Biofoundries see life sketched out in CAD software, designs sent to a robot in another room to assemble, and machine learning applied to the results.

"It's changed dramatically since I started my career!," Keasling offers enthusiastically.

"When I started, the tools were so rudimentary and it took so long to get through the design, build, test cycle – it might change an entire PhD thesis to get around it."

Machine learning is becoming "really hot", and while it's still early days, Keasling sees it as having huge potential in terms of learning from successes and failures.

Are things getting nearer to the predictability levels in electronics engineering, though?

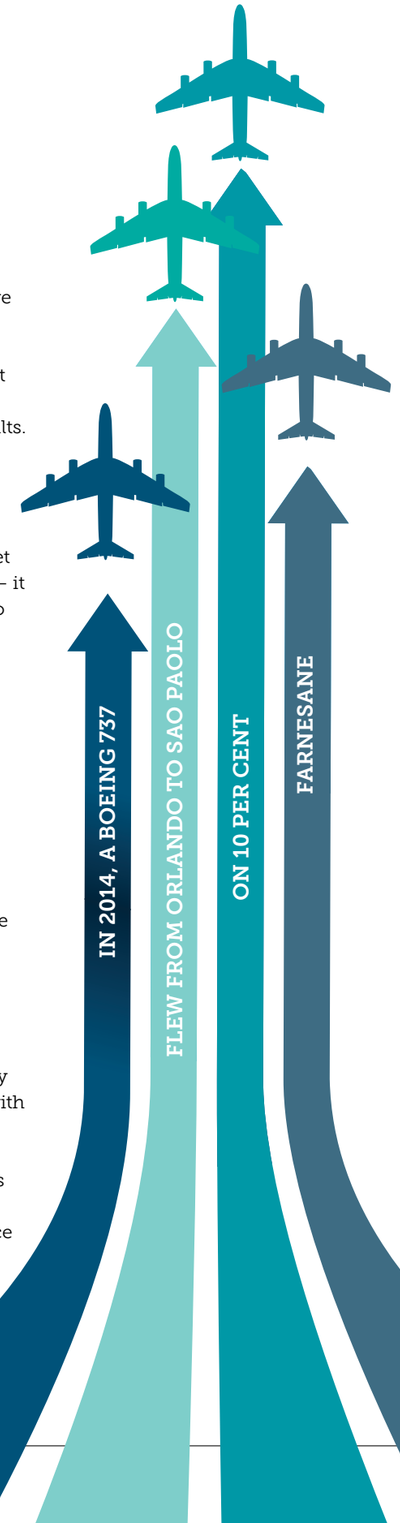
"It's getting closer all the time but we've got a long way to go," answers Keasling. "But that's good. It keeps me in business."

Fuels and the future

In July 2014, a Boeing 737 flew from Orlando to Sao Paolo on 10 per cent farnesane (produced using genetically engineered brewer's yeast) blended with jet fuel.

Biofuels aren't competitive with low-margin diesel or petrol, but this is less the case for aero fuels.

"I think right now, because the price of oil is so low, it's very hard for anything to compete with oil," offers Keasling, who is also CEO of the US Department of Energy's Joint BioEnergy Institute.



"We'll have the best chance of competing with bio fuels within the area of jet fuel because it's a higher priced thing."

Keasling's work concerns drop-in hydrocarbon biofuels, rather than ethanol, which he likes to say is good "for drinking, not for driving". Ethanol cannot use the existing infrastructure used by the fossil fuel industry (such as pipelines and tanker trucks) or be substituted in vehicles.

Early synbio companies such as LS9, Solazyme and Amyris (which Keasling co-founded) set out trying to produce sustainable fuels within their little chemical factories, but success was elusive. Several of them ended up changing course to produce higher-margin specialty chemicals for industries such as cosmetics or food manufacturing.

Amyris's second project after antimalarial medicines was farnesene, sold under the name Biofene. The company describes this as a "renewable hydrocarbon building block", made out of cane sugar by the reprogrammed yeast. Hydrogenated, it makes a diesel drop-in fuel (farnesane), and has been approved to be used as a blendstock in jet fuels.

The production cost of farnesene has come down considerably since the

company began, from $US12 a litre at the beginning of 2013, to $US4 a litre later that year, and then $US1.75 in September last year.

Though production is getting cheaper, for a company like Amyris to be competitive with makers of fossil fuels, carbon penalties would be needed.

"If you burn petroleum-based fuels in your car or your plane or whatever, it goes into the atmosphere, nobody has to pay anything for that and it's causing an environmental collapse," says Keasling.

"If there was a real tax on carbon, Amyris's diesel might be cost-competitive. So it's kind of an unfair playing field, is what I'm saying."

Despite his concerns about the sustainability of current approaches towards conventional fuels, the chemical engineer is optimistic about the future, and about what can be accomplished with good old *S. cerivisiae*.

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At the mention of the subject, he confesses his excitement again, and reminds us that we're just getting started when it comes to exploiting the ancient microbe. With ever-more powerful tools and the right minds on the task, things just might be okay.

"I think there's huge potential in terms of engineering yeast and we're just seeing the potential right now, but I think there's so much more to do," says Keasling.

"I'd like to see us turn brewer's yeast into something known to be a hydrocarbon overproducer." ●

ALL ABOUT ARTEMISININ

In 2005, the World Health Organisation recommended artemisinin-based combination therapies as the best way to treat malaria.

Previously treated by quinine and chloro-quinine, the malaria parasites have long since become tolerant to these. Combination therapies are recommended to reduce the chance of plasmodium developing a tolerance to artemisinin, which is effective in about 95 per cent of cases.

Works identifying the antimalarial medicinal properties of artemisia annua, or sweet wormwood, date back to 168 BC. Its efficacy was forgotten, then rediscovered during the Vietnam War by Youyou Tu, a traditional Chinese medicine specialist. Last year she received a joint Nobel Prize for her discovery.

"Her work is really phenomenal and she's saved millions of lives; definitely deserved that award," says Jay Keasling.

According to WHO, malaria killed roughly 438,000 in 2015. Ninety per cent of victims were in the African region, and were mostly children.

Though Youyou's discovery is the most effective known way to kill the plasmodium parasite, it is not universally available. There have also been shortages as well as wild price fluctuations for the medicine.

In 2001, one of Keasling's graduate students found a paper on artemisinin, and suggested it might be synthesised synthetically. E. coli was the first 'chassis' or microbial host used, with a paper published on this in *Nature Biotechnology* in 2003.

Yeast ended up being a better option with more favourable internal machinery, including the cytochrome P450 enzyme in its metabolic pathway.

The eventual process is for artemisinic acid, a precursor material, as artemisinin itself is toxic to yeast as well as to the plasmodium. Following years of development to increase yields assisted by two grants totalling US$53 million from the Bill and Melinda Gates Foundation, industrial production of semi-synthetic artemisinin began in 2013.

The technology is licensed to Sanofi, which produces combination therapies on a 'no profit, no loss' basis (a level of about US$350 to US$400 per kg) to level out spikes in demand. (It produced no semi-synthetic artemisinin last year, due to an oversupply at the time).

In April this year, the Gates Foundation invested US$5 million in Amyris to fund R&D to further decrease the cost of production.