FOCAL POINT

SYNTHETIC BIOLOGY IN JAPAN

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Smart Cell Industry

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FOCAL POINT

SYNTHETIC BIOLOGY IN JAPAN

BIOLOGY BUSINESS

Countries are pouring research funding into industrial production that uses microbes and plant cells. Some predict these will ultimately produce up to 60% of the global economy's physical inputs. Many systems will make use of fermentation, an area of Japanese research strength. A national initiative in Japan called the Smart Cell Project is launching a suite of new technologies to accelerate the development and commercialization of bio-production methods.

FOCAL POINT ON SYNTHETIC BIOLOGY IN JAPAN

PARTNER CONTENT

FOCAL POINT ON SYNTHETIC BIOLOGY IN JAPAN

PRODUCED IN PARTNERSHIP WITH THE NEW ENERGY AND INDUSTRIAL TECHNOLOGY DEVELOPMENT ORGANIZATION

A FOUNDATION OF FERMENTATION

With biotechnology set to drive sustainable new chemistry processes, Japan is banking on its history of fermentation research and a well-funded 'BIOFOUNDRY' to push ideas to commercialization.

In Japan, harnessing the power of microbes is nothing new. Staples, including soy sauce, some pickles, sake and miso, are all produced using fermentation. And Japan already has one of the largest probiotics markets in the world.

Tomohisa Hasunuma, who leads microbial research for one of Japan's largest biotechnology research efforts, the Smart Cell Project, says its long history with fermentation puts Japan in a leading position for the chemistry of the future: engineering plants and microbes to produce the base materials for everything from drugs to fuels, a major goal in the field of synthetic biology.

Global consulting firm McKinsey reported in May 2020 that biology could ultimately produce 60% of the physical inputs to the global economy. Such conclusions are underlined by a report from the European Commission suggesting the European Union could replace roughly 30% of products derived from petroleum by 2030, using biotechnology.

Bioeconomy future

A global 'bioeconomy' driven, at least partly, by biology-based production is perhaps only a decade away, according to John Cumbers, a Silicon Valley investor and former synthetic biologist at NASA's Ames Research Center.

He points to Spiber, a Japan-based start-up that is fermenting microbes to produce synthetic material designed to emulate the durability and flexibility of spider silk. "The advantage here is that

LIVING FACTORIES
In the short-term, microbes will likely drive biological production. Even though plants produce more than a million kinds of secondary metabolites, Takeshi Matsumura explains that engineering plant cells is more complex. He thinks that big data on plant metabolic pathways will help construct key artificial pathways in microbes. Japanese researchers are also developing tools to turn on and off gene expression in plant cells (below) for productivity gains.

the performance properties are better than silk," explains Cumbers. Working with American outdoor recreation outfitter, The North Face, the Smart Cell Project-funded Spiber produced the first commercially available jackets using the synthetic silk late last year.

Satoru Kuhara, who works for the Japanese innovation funder, the New Energy and Industrial Technology Development Organization (NEDO), leads the Smart Cell Project. He says the bioeconomy movement is driven by both economic and environmental imperatives.

Bioplastics, for example, can minimize the role of petroleum in production and then break down into water and carbon dioxide when exposed to microorganisms in soil.

The Japanese corporation, Mitsubishi Chemical, already uses microbes to help produce a substitute for polyethylene, one of the most widely available plastics in the world, often formed into shopping bags and long sheets of mulch film for agricultural purposes. To produce the bioplastic, gene-edited bacteria ferment biological material, and help to create one of the base materials used to make the bioplastic polymer polybutylene succinate.

Biofoundry test bed

Bioplastics, however, have not yet replaced traditional plastics globally. Biology-based products, explains Cumbers, often have to compete with trillions of dollars of investment in petroleum-based infrastructure.

While there are more than 400 synthetic biology product start-ups in the United States, the numbers across Asia are still fairly low. A number of Smart Cell Project-funded labs, including a biofoundry at the Engineering Biology Research Center at Kobe University, will help address this by speeding up proof-of-concept work for companies. The Kobe-based biofoundry is filled with new, automated research tools that have been in development since 2016 through the Smart Cell Project. To date, 40 companies have partnered on development.

One example of the new technology powering the foundry is based on the work of Kenji Tsuge, who published in *Scientific Reports* in 2015 on a system that assembles an unprecedented number of DNA fragments (more than 50) in one step.

The Smart Cell Project has since automated the insertion of this system into cells. Enabling the introduction of a large amount of DNA in one action has addressed a longstanding need for faster and more accurate DNA editing to create new functions in living cells, explains Kuhara.

Other innovations include machine-learning tools capable of identifying useful biosynthesis pathways; an automated pre-treatment system for metabolomic analysis; and, an enzyme engineering analysis system to improve production titers. Research on new engineering tools continues, adds Kuhara.

At the biofoundry, Hasunuma's team has already started work on some widely useful compounds, including alkaloids such as reticuline, which can be used to produce pain medication.

In 2019, Hasunuma's group improved alkaloid titers by more than 10-fold by narrowing a metabolic pathway in *Escherichia coli* using new enzyme shortcuts. He says that the alkaloids are now being produced at almost commercially viable levels.

Hasunuma's ambitious goal is "being able to produce anything". While that's some way off, he thinks the biofoundry will provide viable, scalable, biology-based production systems to partners within two years. The hope, he says, is that this evidence-based development, a strong fermentation research foundation, and a general momentum will push Japan into a leadership position in the global bioeconomy. ■

1

FERMENTATION, which, among other things, is used to make glutamate to create *umami* in Japanese soups, is a key production process for microbes.

2

Classic targets of Japan's fermentation research include **CENTRAL METABOLITES,** such as glutamate and succinate. Polybutylene succinate is a useful bioplastic.

3

In 2018, the European Commission detailed bioplastics research as part of an environmentally sound **ECONOMIC INVESTMENT STRATEGY**. Satoru Kuhara agrees that bioeconomies will be more sustainable.

Satoru Kuhara leads the **SMART CELL PROJECT**.

The bioeconomy movement is driven by both economic and environmental imperatives.

IMPROVED GENE-EDITING PRECISION TO BOOST JAPAN'S BIOECONOMY

A GENOME-EDITING TECHNIQUE CALLED TYPE I-D (TiD) is based on a lesser-known CRISPR-Cas system and will help engineer the cells that drive the biology-based production of raw materials in Japan.

To kick-start a Japanese bioeconomy in which raw materials are produced using gene-engineered cells, researchers will require access to sophisticated, locally owned CRISPR-Cas-based genome editing tools. A number of teams linked to Japan's Smart Cell Project have been developing these tools since 2016, as part of a national effort to build the research ecosystem needed to commercialize industrially productive cells.

"We want to improve the range and accuracy of available tools, build user-friendly packages, and avoid costly intellectual property issues," says Takahiro Nakamura, a plant molecular biologist from the Faculty of Agriculture at Kyushu University. His group are tasked with working on RNA editing techniques, tool delivery and the intellectual property of the project's genome editing tools.

CRISPR type I-D editing tool

A new tool was recently discovered by a team at Tokushima University's Graduate School of Technology, Industrial and Social Sciences. The tool, dubbed type I-D (TiD), is based on the lesser-known type I CRISPR system.

CRISPR-Cas tools are of two different classes, consisting of six systems and at least 34 subsystems. Globally, many teams are studying the well-characterized systems involved in CRISPR-Cas9, CRISPR-Cpf1 and CRISPR-Cas3, but many CRISPR families are yet to be fully explored as genome editing tools, says Nakamura.

Tokushima University researchers developed their tool by examining Cas effector proteins in the lesser-known type I CRISPR system, says Keishi Osakabe, who leads the group that developed TiD.

CRISPR systems comprise two components: a guide RNA, which is a short RNA fragment essential for complementary binding to the target segment of the genome, and CRISPR-associated (Cas) proteins, which are capable of snipping off targeted DNA fragments near the binding site.

The type I CRISPR systems have some advantages in functionality, explains Osakabe, including longer guide RNA sequences and different mutation profiles.

Typical guide RNAs are about 20 nucleotides long for CRISPR-Cas9 tools, but the TiD system typically uses 35- or 36-base guide RNAs, says Osakabe. Their length may help mitigate off-target effects, one of the main challenges limiting CRISPR technology's practical and commercial potential. The longer guide sequences could potentially be more effectively customized to accurately identify a target sequence, lessening the risk of mutations, deletions, insertions, inversions and translocations.

LONGER GUIDE SEQUENCES COULD POTENTIALLY BE MORE EFFECTIVELY CUSTOMIZED.

The DNA cleavage mechanism is also unique to other common systems, says Osakabe. Cleavage is performed by a specific Cas protein, such as Cas9, Cpf1, and Cas3. However, unlike other CRISPR systems, TiD's Cas10d protein is involved in stabilization, the recognition of the cleavage site, and as the functional nuclease that splits DNA molecules. As a result, TiD can induce both bi-directional long-range deletions and short insertions/deletions. The ability of type I CRISPR to generate such a diverse range of large deletions from a single targeted site could potentially enable long-range chromosome engineering that would allow simple, fast and effective multi-gene function screening studies, says Osakabe.

The potential applications of TiD are being further developed by a multidisciplinary team from Tokushima University, RIKEN, Meiji University, and Kindai University.

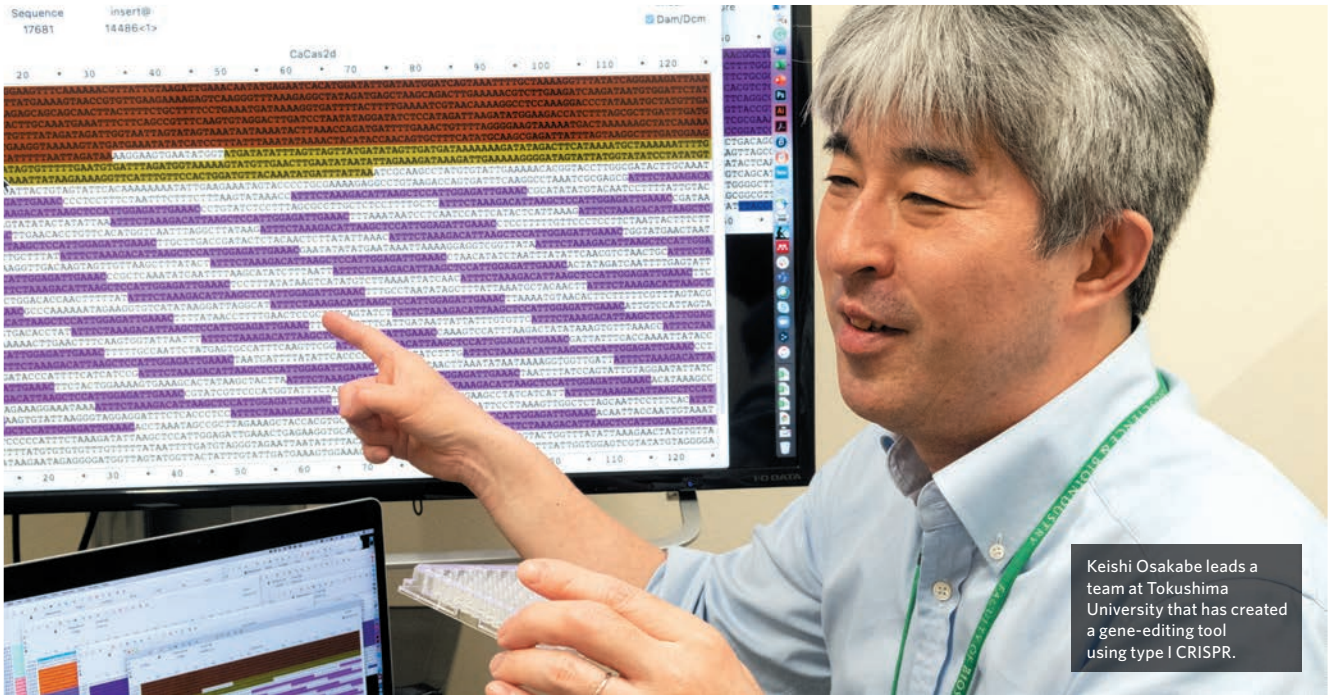
Packaged and delivered

The team refer to these engineered cells as 'smart cells' and Nakamura heads one of the Smart Cell Project's other groups currently looking at how to package genome editing tools. He brings experience in developing RNA editing techniques using plant proteins for a successful Japanese start-up, and he points out that, for industry, "it's important to establish user-friendly packages".

To this end, Nakamura aims to establish new base recognition, editing, and delivery techniques for Japanese gene-editing technology. His group has focused on accurate DNA recognition modules, and

Tokushima University researchers harnessed cutting-edge bioinformatics and genetic engineering.

A researcher working on a new gene-editing tool, type I-D (TiD).



Keishi Osakabe leads a team at Tokushima University that has created a gene-editing tool using type I CRISPR.

effective and easily assembled delivery systems for technologies such as TiD.

Nakamura emphasizes that the combination of effective research and real-world savvy will be important to drive interest from industry and develop the right collaborations to harness the current global momentum towards cell-driven production. "Japan's smart cell industry will thrive if it can harness a series of innovative biotechnologies for bioinformatics and metabolomics developed by other Smart Cell Project participants that integrate DNA sequencing, artificial intelligence and machine learning for efficient bio-design."

For this to work, adds Osakabe, new genome editing tools, such as TiD, will be essential to engineer target cells quickly and reliably. This will enable proof-of-concept testing for cell systems identified by the project's other new tools, he says.

"The convergence of the accomplishments in the Smart Cell Project, including novel genome-editing tools, will be a key feature in its success and the acceleration of the bioeconomy market," he says.

Osakabe's group has already started to apply TiD to plants to increase the production of high-function biomaterials.

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

Tokushima University
www.tokushima-u.ac.jp/english/

Green fluorescent protein expression was used to test a genetic ON/OFF mechanism in herbaceous plant *Nicotiana benthamiana*.

THE PRODUCTIVE PLANT SWITCH

Synthetic biology is being used to genetically engineer flora to produce industrially useful products, all with ON/OFF control.

To more reliably produce industrially useful plant-based products, Hiroshi Masumoto and Daisuke Shibata, from the Kazusa DNA Research Institute in Japan, are inserting strings of genetic instructions controlled via protein packages.

Though scientists have now become adept at introducing genes into plants, adding more than a handful is still very difficult, as is precisely controlling the expression of these introduced genes. Both challenges limit the ability to engineer healthy plants to produce specific chemicals for industry, say the researchers.

Masumoto and Shibata are harnessing technology usually used to manipulate artificial chromosomes for clinical research to generate and insert long stretches of synthetic, controllable DNA into plant genomes.

"We are focusing on creating isopentenyl diphosphate (IPP), which is an important precursor to other metabolites, including natural rubber, and the starting compounds for the biosynthesis of anti-cancer drugs or novel biopolymers, such as polyterpenoid resins," explains Masumoto.

NOW THEY ARE INSERTING LONG STRETCHES OF SYNTHETIC, CONTROLLABLE DNA INTO PLANT GENOMES.

In collaboration with Seiji Takahashi from Tohoku University, an expert on IPP metabolism, the team from Kazusa DNA Research Institute is building a stretch of DNA to carry all seven genes in the IPP synthesis pathway.

Once complete, these seven genes will be inserted into plants, and will be able to be manipulated by bacteria-and-plant-protein fusion packages. These packages can control gene expression by changing how the inserted genes are placed into a DNA packaging structure known as chromatin.

The shape of DNA's chromatin wrapping affects how strongly a gene is expressed. So when a modified plant is treated with a fusion package through its leaves or root system, the bacterial portion binds to engineered sites on the inserted genes and the plant portion can cause the chromatin around the inserted genes to close, limiting gene expression.

Treating plants with different fusion-protein packages can also reverse this process. "This way we can shut down all the introduced genes when they are not needed and then switch them all on at once," explains Shibata.

The ability to deactivate inserted genetic instructions is an advantage over existing genetic engineering approaches, which can sometimes result in imprecise results. For example, if some of the introduced genes switch on at the wrong time, it can cause unintended consequences – such as inhibited growth, silencing relevant gene expression, and unhealthy plant specimens.

According to Shibata: "Further development of this research should help plants, or microbes, reliably produce many industrial substances. We hope the technology will be used widely in Japan's future bioeconomy."

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

Kazusa DNA Res. Inst.

www.kazusa.or.jp/en/

Giving the green light to useful plant genes

A new technique uses VIRUS-BORNE ENZYMES to control a METHYLATION-BASED ON/OFF SWITCH for plant genes.

A demethylated tobacco plant (top) displayed fluorescence after silencing genes were turned off. A control plant (bottom) shows the plant's typical appearance.

A new technique is thought to be the first to precisely control methylation-driven gene deactivation in multiple generations of plants, says Takeshi Matsumura, a collaborator on the research from the National Institute of Advanced Industrial Science and Technology in Japan.

"Refining the technology will mean that researchers can tweak plant gene expression rather than cutting or inserting new bits of DNA," explains Matsumura. This form of genetic engineering, he says, may help side-step some gene-engineering issues, such as the corporate ownership of key crop strains.

Methylation turns genes on and off in plants by adding chemical tags to segments of DNA; this tagging is guided by RNA molecules. With Matsumura, plant pathologist, Chikara Masuta, from Hokkaido University is developing enzymes that target and cleave these RNA guides, stopping the specific sequences of genes from being silenced.

Plants are innoculated by gently rubbing material containing engineered viruses on their leaves. Once infected, a type of RNA enzyme known as a virus ribozyme inside the plant cells precisely seeks, snips and destroys key RNAs. The end result: "We can specifically erase some types of methylation," explains Masuta.

During proof-of-concept testing, the technique was used to stop plants from turning off fluorescence genes, making the test plants glow green (left). Some plants passed the loss of methylation on to their progeny, indicating that the treatment effect can last through the generations, to some extent, says Masuta.

The researchers have also been able to use the method to stop the deactivation of a transposon — a DNA-modifying element that can have an epigenetic effect. Turning on transposons will be useful to researchers exploring the function of genes, say the researchers.

Rosmarinic acid to start

With a plan to boost yields of rosmarinic acid, a bioactive compound touted for its anti-oxidant and anti-inflammatory properties, Masuta and Matsumura are now attempting to apply their technique to shiso plants, a natural source of the prized molecule.

The researchers performed many of these initial experiments in wild tobacco plants. "But," says Masuta, "I really feel that this technology is applicable to any species".

The hope, he says, is to eventually use the epigenetic technique to help create greenhouses full of plants that pump out complex pharmaceuticals, nutraceuticals, and other high-value plant-derived compounds.

The team are discussing other genes, compounds and plants of interest with biotech companies. "We hope this will become a globally used technique for engineering plants that can produce all kinds of secondary plant metabolites at commercial scales," Matsumura says.

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

Masayuki Inui next to an octuple jar fermenter producing usable chemicals, such as catechol, by fermenting an engineered strain of bacterium, *Corynebacterium glutamicum*. The system controls temperature, pH and oxygen levels.

DESIGNER METABOLIC PATHWAY FOR SUSTAINABLE AROMATICS

Recombinant bacteria fermenting in bioreactors are producing USEFUL QUANTITIES OF CATECHOL, a substance used in the production of fragrance chemicals and pharmaceuticals.

To help meet Japan's greenhouse gas emissions targets by 2030, scientists at the Research Institute of Innovative Technology for the Earth (RITE) are developing bacteria to produce commercial quantities of industrially important aromatics.

One of the most promising aromatic compounds RITE has been able to create is catechol, widely used as an intermediate in the fragrance and pharmaceutical industries. Catechol is highly toxic to microorganisms and was thought to be impossible to create using fermentation technology.

RITE researchers are currently producing world-leading catechol levels using a non-pathogenic soil bacterium, *Corynebacterium glutamicum*. While the bacterium doesn't yield catechol naturally, it has an inherently sturdy cell wall, so is quite resistant to chemical stress, explains Masayuki Inui, who leads the Molecular Microbiology and Biotechnology Group at RITE.

"So, we constructed an optimized metabolic pathway in the bacterium and inserted the genes for a catechol-catabolic enzyme," he explains.

WE MANAGED TO ACHIEVE THE HIGHEST PRODUCTION DENSITY OF CATECHOL IN THE WORLD TO DATE.

He says this couldn't have been done without a new Japanese metabolic design system developed as part of a push to create a market driven by microbial fermentation. Alongside other omics data, it enabled efficiencies, such as optimum metabolic pathways, genetic sequences, and regulatory networks.

"Suggestions for multiple modifications were mounted into a single microbial strain," says Inui. "Using each of these systems, we managed to achieve the highest production density of catechol in the world to-date."

Teams at RITE have also optimized *C. glutamicum* strains to produce organic acids and aromatic compounds such as shikimic acid and phenol, both widely used to manufacture drugs, cosmetics and plastics.

Bioreactors for business

In tandem, RITE researchers have developed an efficient microbial production process. In it, their engineered microbes are densely packed into bioreactors that promote fermentation. Deprived of oxygen, the microbes stop growing. However, the microbes' major metabolic pathways remain active, allowing them to ferment useful products without additional growth medium or external energy.

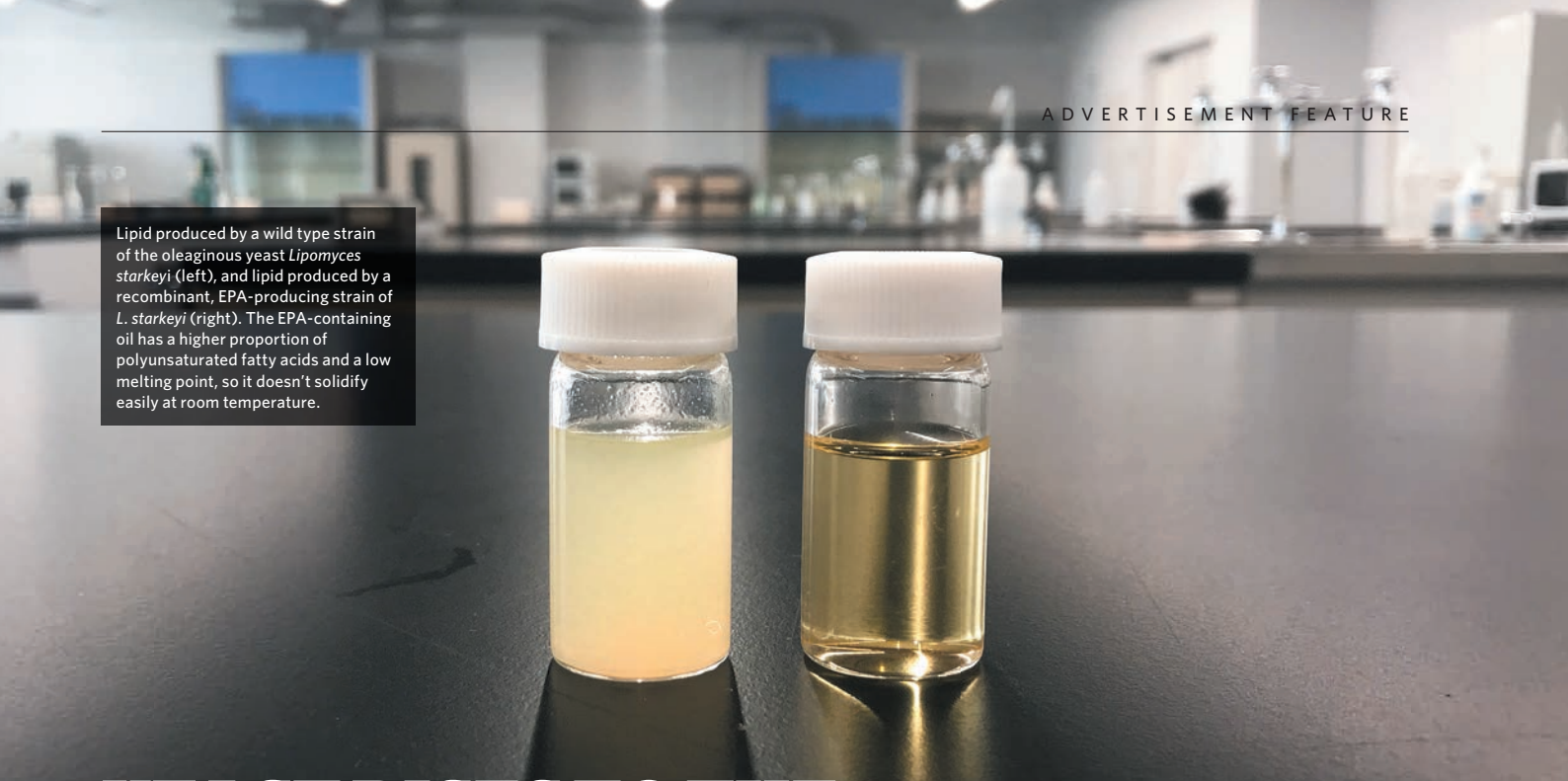
"We also hope to create production processes that use sequestered CO_2 from other industries as a fermentation substrate, so that these processes can also act as a carbon sinks, lowering the concentration of CO_2 in the atmosphere," he adds.

"Based on our success in catechol production, we believe that our metabolic design system can be applied to synthesize many other petroleum-derived aromatic compounds," Inui says. "And by developing and disseminating fermentation technology that uses renewable resources, I believe we can move from being dependent on fossil resources to a more sustainable society in the next decade."

The research and metabolic design system described are part of Japan's Smart Cell Project, run by the New Energy and Industrial Technology Development Organization (NEDO). ■

RITE
Research Institute of Innovative Technology for the Earth

www.rite.or.jp/en/



Lipid produced by a wild type strain of the oleaginous yeast *Lipomyces starkeyi* (left), and lipid produced by a recombinant, EPA-producing strain of *L. starkeyi* (right). The EPA-containing oil has a higher proportion of polyunsaturated fatty acids and a low melting point, so it doesn't solidify easily at room temperature.

YEAST RISES TO THE OMEGA-3 CHALLENGE

Hopes are high that METABOLICALLY ENGINEERED *LIPOMYCES STARKEYI* might provide a sustainable source of complex omega-3 polyunsaturated fatty acids.

The last decade has brought increased demand for omega-3 polyunsaturated fatty acids (PUFAs) as a component of infant food formula, dietary supplements and aquaculture feed. This has placed pressure on major sources of omega-3 PUFAs, fatty fish, such as salmon and mackerel. Now researchers at Niigata University of Pharmacy and Applied Life Sciences in Japan (NUPALS) have engineered a yeast as an alternative source.

It's already been shown that microalgae can produce PUFAs, such as eicosapentaenoic acid (EPA). But microalgae face mass-culture production obstacles and levels of lipid production that are too low. NUPALS researchers have now engineered a yeast, *Lipomyces starkeyi*, to produce EPA, says Hiroaki Takaku, who led the research at NUPALS.

L. starkeyi is one of several so-called oleaginous yeasts, which can accumulate large amounts of lipids in the form of triacylglycerol. "*L. starkeyi* has a higher lipid content than other oleaginous yeasts and can accumulate up to 85% of its dry cell weight," explains Takaku. "It has a poor ability to degrade its own lipids, which is a great advantage."

RESEARCHERS HAVE NOW ENGINEERED A YEAST, *LIPOMYCES STARKEYI*, TO PRODUCE AN OMEGA-3.

However, *L. starkeyi* only synthesizes up to 18-carbon long PUFAs, says Takaku. "So, we introduced a part of an algae's EPA synthesis system to give it the ability to produce EPA, a 20-carbon PUFA."

Then the researchers began to look for efficiencies. Production of EPA is a multi-stage process requiring a succession of actions by elongase and desaturase enzymes. Using a knowledge-based machine learning method, developed by Michihiro Araki at Kyoto University, the researchers explored protein databases for candidate eukaryotic microbial desaturases and elongases that would function optimally in *L. starkeyi*.

Takaku's group then transferred multiple recombinant genes for these enzymes into *L. starkeyi* using Japanese-developed long-chain DNA synthesis technology. This *L. starkeyi* produced PUFAs at up to 18.4% of total fatty acid output (10% EPA).

The researchers also used a regulatory network analysis method, developed by Sachiyo Aburatani at the National Institute of Advanced Industrial Science and Technology, to help increase the expression of genes in the fatty acid synthesis pathway, doubling EPA production.

Finally, by comparing the genomes of a natural *L. starkeyi* strain and lipid-accumulating variant strain, a new control factor in lipid production was found. Manipulating it achieved a four-fold increase in lipid levels.

It's remarkable, Takaku says, that a series of Japanese-developed technologies has yielded a yeast that originally didn't produce EPA to reach this output after only two years. He thinks commercial production is only a few years away.

This research and each of the analysis technologies mentioned are part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

Niigata University of Pharmacy and Applied Life Sciences

www.nupals.ac.jp/english/

A systematic approach to scaling up synthetic biology

LEAN AND SMART DATA TOOLS are key to building a robust economy based on products created by engineered plants and microbes.

A series of streamlined computational research tools will help Japanese synthetic biologists produce rapid results for sustainable industries that use living organisms to produce base materials.

This 'bioeconomy' could include everything from fragrances that the incorporate substances metabolized by engineered strains of bacteria to palm oil alternatives produced by yeasts, explains Sachiyo Aburatani, who helps steer the Computational Bio Big Data laboratory at Japan's National Institute of Advanced Industrial Science and Technology.

"Just by trying to substitute 10% of all palm oil to sustainable alternatives, there's already a demand for 60 million tonnes of oil per year," Aburatani points out. "Using substitutes from yeast could lead to as much as a reduction of 240 million tonnes of CO_2 annually."

Japanese synthetic biology has an advantage: the country's history with fermented foods, such as soy sauce and pickles, has seen research strengths in two vital fields — fermentation and microbes. With this foundation and a systematized research approach, scientists expect to make rapid progress toward a bioeconomy over the next 10 years.

Using leaner data

Aburatani has been working on lean data tools, including gene network maps. "International teams are using machine learning to identify genes in an organism that can be modified to help it generate usable amounts of a product. But to do this, you need tens of thousands of data points for each study," she explains. "It's very expensive. Instead, in Japan, we're developing modelling techniques that can achieve similar levels of precision with realistic amounts of data, several hundred data points, at a minimum."

WE REDUCED BY HALF THE AMOUNT OF TIME IT TAKES FOR BULK DNA SYNTHESIS, AND THE COST TO A TENTH.

For example, with statistical methods, Aburatani constructs her network maps to estimate the cause and effect between genes that contribute to the production of a compound. "When a gene is in action, it switches other genes on and off, creating a very complicated and precise control network system within an organism," she explains. "If this network can be reconstructed, a useful control factor to adjust the target substance can often be discovered."

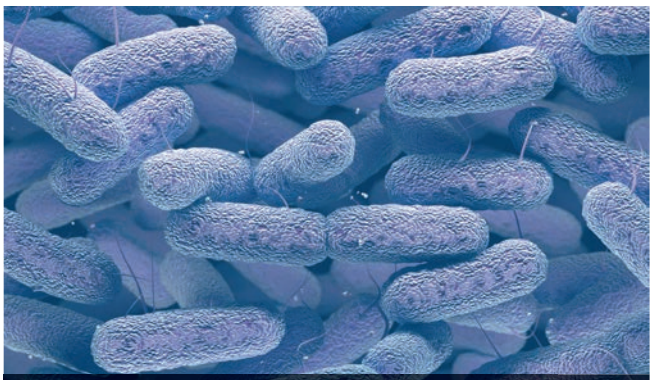
In an experiment using this computational technique, Aburatani found a gene, in which the function was previously unknown, that contributes to doubling oil yield in yeast from the *Lipomyce* genus. Once systems like this are revealed, says Aburatani, the necessary data needed to make discoveries in similar areas should be reduced by tenths, or even hundreds.

Bio-production pathway tools

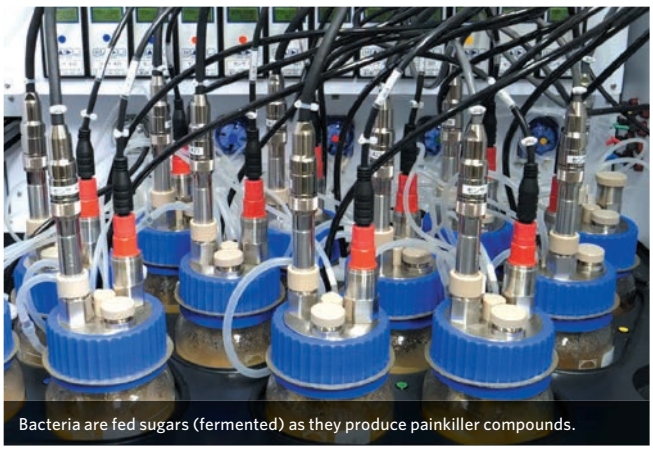
Tomokazu Shirai, who leads the Cell Factory Research Team at RIKEN, is designing artificial metabolic pathways. His team has established a computational tool called BioProV that pulls data from enzyme databases to find reactions that could comprise a new route.

He says cells are often modified based on computer reconstructions of their metabolism that predict how their product yield would change after editing a specific gene. But Shirai thinks this isn't enough. "To make an entire industry, we also need to take the opposite approach, where we start from a desired product and find a way to make it," he says.

With BioProV Shirai's team can suggest the insertion of



A silkworm enzyme was inserted into *Escherichia coli*'s metabolism to increase the output of tetrahydropapaveroline and reticuline by more than a factor of seven each.



Bacteria are fed sugars (fermented) as they produce painkiller compounds.

candidate genes into a microbe or plant cell that codes for enzyme catalysts that correspond to desired chemical reactions. "This way, we can construct a pathway to synthesize non-natural compounds using enzymes an organism doesn't naturally produce," he explains.

Shirai's group also harness a tool called M-Path, developed by Michihiro Araki at Kyoko University, to analyse metabolic reactions and design new metabolic pathways. These tools, he says, expand the repertoire of possible modifications. "We're no longer limited to optimizations on enzymes and reactions that already exist."

Accelerating production

Tomohisa Hasunuma, who leads the Engineering Biology Research Center at Kobe University, is using tools like M-Path to enhance real-world outputs.

"Our group is working to make new metabolic designs a reality through automated processes, so that high-performing cells can be produced in a shorter time," he explains.

So far, his team has established the machinery to synthesize long chains of DNA, which are inserted in cells to bulk-edit genes. "At the moment it's the most precise tool of its kind. We reduced by half the amount of time it takes for synthesis, and the cost to a tenth."

His group is working on two compounds that can be used to manufacture painkillers, tetrahydropapaveroline and reticuline. With insights from M-Path and an iterative breeding and research process called the design, build, test, and learn (DBTL) cycle, his team increased the microbial titres of tetrahydropapaveroline by up to a factor of eight and reticuline by more than a factor of seven.

This was achieved after M-Path calculations showed that an enzyme found in silkworms could be used as a more efficient alternative for two bacterial enzymes, a metabolic shortcut. The team then artificially fine-tuned the silkworm enzyme to fit more naturally within the *Escherichia coli* bacterium's metabolism.

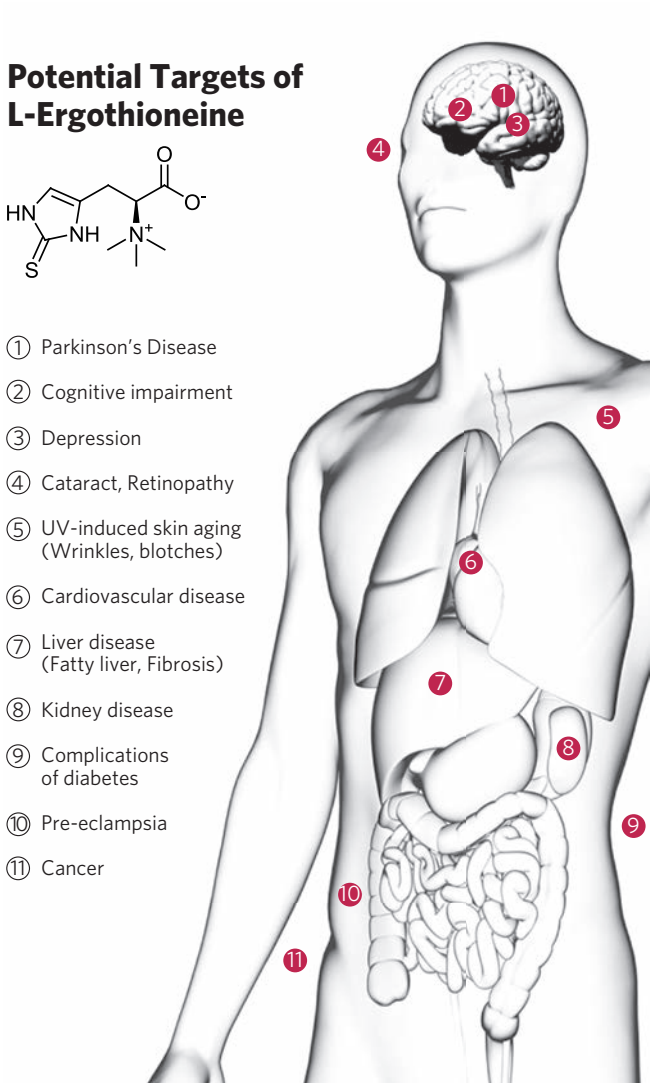
"We've made tremendous progress in metabolic design and automated cell culture techniques, and finally have all the research components to breed efficient bacteria using the DBTL cycle," says Hasunuma. "This breeding will be a crucial next step to scale up the production process and establish a competitive industry in which target compounds can be mass produced."

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

New Energy and Industrial Technology Development Organization
www.nedo.go.jp/english/index.html

Breakthrough in bio-based production of longevity vitamin, ergothioneine

OPTIMIZED FERMENTATION OF ENGINEERED MICROBES may be the solution to mass producing a vitamin-like antioxidant with anti-aging properties.



Recent Japanese research suggests that fermentation using engineered microorganisms may bring the production costs of ergothioneine, a vitamin-like antioxidant closely linked to longevity, down by up to 99%.

Ergothioneine is part of a group of compounds dubbed 'longevity vitamins' by the likes of Bruce Ames, a well-known expert in ageing and professor of Biochemistry and Molecular Biology Emeritus at the University of California, Berkeley. It's argued that these compounds may form a new group of vitamins, as they may be essential to long-term health and must be absorbed through diet. Ames described this theory in *PNAS* in 2018. Recent studies show that ergothioneine eliminates many reactive oxygen species and may slow the development of wrinkles, frailty and cognitive decline in aged populations, explains Takeshi Nakatani, a lead researcher at Japanese chemicals trading firm, NAGASE & CO., LTD. Low levels of ergothioneine have also been linked to mild cognitive impairment, Parkinson's disease, cataracts and cardiovascular disease.

"Ergothioneine is made by mushrooms and a few microorganisms, but the human body has a mechanism that specifically imports and accumulates ergothioneine in cells and organelles susceptible to oxidative stress and inflammation, such as the brain, skin, eyes and liver," says Nakatani. The 2005 discovery of this transporter made it a hot topic of research, but industrial development has been limited by the compound's cost.

When ergothioneine research kicked off at NAGASE in 2014, a kilogram cost nearly US$1 million. "The amount in mushrooms is negligible, and organic synthesis has an environmental impact, so neither meets our sustainability goals," Nakatani adds. He says that by modifying biosynthetic pathways in bacteria and optimizing fermentation, his team was recently able to increase production efficiency by a factor of approximately one thousand.

Leading production levels

NAGASE's involvement in the Smart Cell Project, a Japanese effort to encourage production using microorganisms and plant cells, has been critical. Research partnerships, says Nakatani, have given them access to cutting-edge enzyme design and metabolic analysis technologies. "As a result, I think we're looking at productivity many times higher than recently reported by other Japanese and European teams," he says.

Recently, NAGASE, along with research partners from the Smart Cell Project, also identified genes associated with pumping out ergothioneine through cell membranes. "A crucial aspect is to get the cell to export the compound so that we can efficiently process it downstream," Nakatani explains.

"Now it's about scaling up to industrial production," he adds. Adjustments to cell design continue; NAGASE's own high-throughput system is facilitating optimizations as it evaluates several thousands of cell lines each week. The firm is in talks with businesses in the food, cosmetics and pharmaceutical industry.

This research is part of Japan's Smart Cell Project, which is run by the New Energy and Industrial Technology Development Organization (NEDO). ■

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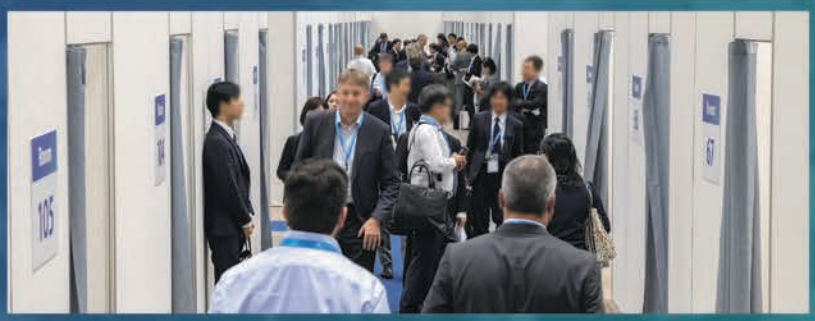

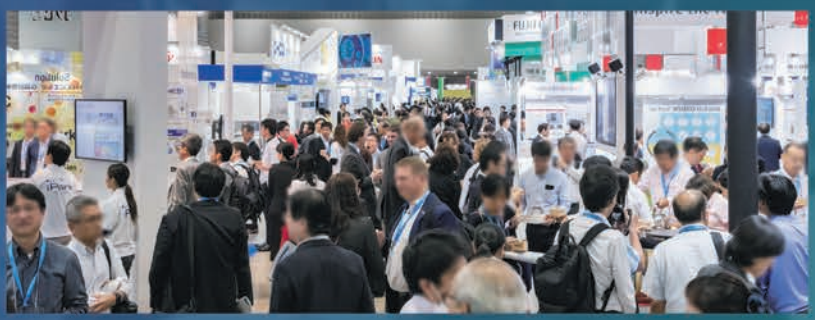

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