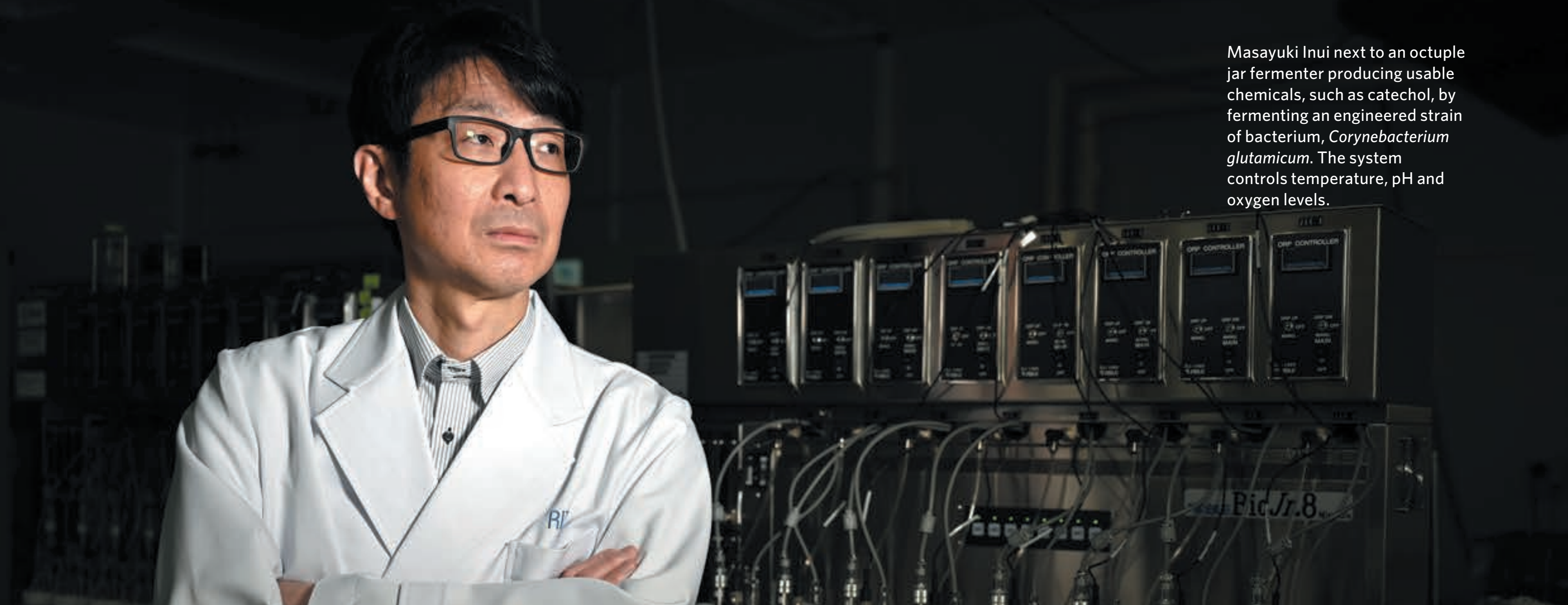

Masayuki Inui next to an octuple jar fermenter producing usable chemicals, such as catechol, by fermenting an engineered strain of bacterium, *Corynebacterium glutamicum*. The system controls temperature, pH and oxygen levels.

# 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

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