Smart Cell Project Achievements

Development of “made-in-Japan” innovative biotechnologies for efficient production of industrial materials using plants and microorganisms

国立研究開発法人 新エネルギー・産業技術総合開発機構
New Energy and Industrial Technology Development Organization

Introduction

Trailblazing a future with bio and digital

The “Development of high-functional product production technology applying organisms such as plants” (smart cell project) will reach a milestone as a five-year project at the end of 2020. Through the fusion of bio and digital technology, this project has developed new approaches and various tools to efficiently extract “potential biological functions” that were previously unavailable. By applying these tools to collect and analyze biological information, and modify and express biological functions, high expectations exist regarding the development of a bio-economy that sets forth significant changes in the economy and society. There are hopes that various industries and academia will utilize these tools to realize a society filled with circulatory bio-derived products.

"Project for Development of Production Techniques for Highly Functional Biomaterials Using Smart Cells of Plants and Other Organisms“
Project Leader : Dr. Satoru Kuhara
Kyushu Univ., Professor emeritus

Reliable measures towards the realization of a bio-economic society

Through the five-year project and the development of an array of technology required for improvement of host productivity and optimization, which are issues for substance production applying biological functions, a wide range of results is considered to have been achieved, from basic technologies that should be put to practical use in Japan, to those that can be applied and implemented in a relatively short time based on accumulated validation cases of corporate themes.

Going forward, the selective use of results from this project, according to the development stages of the companies/research institutions, knowledge in many business fields will be further accumulated, refined as practical technology, and realized in Japan's bioeconomic society.

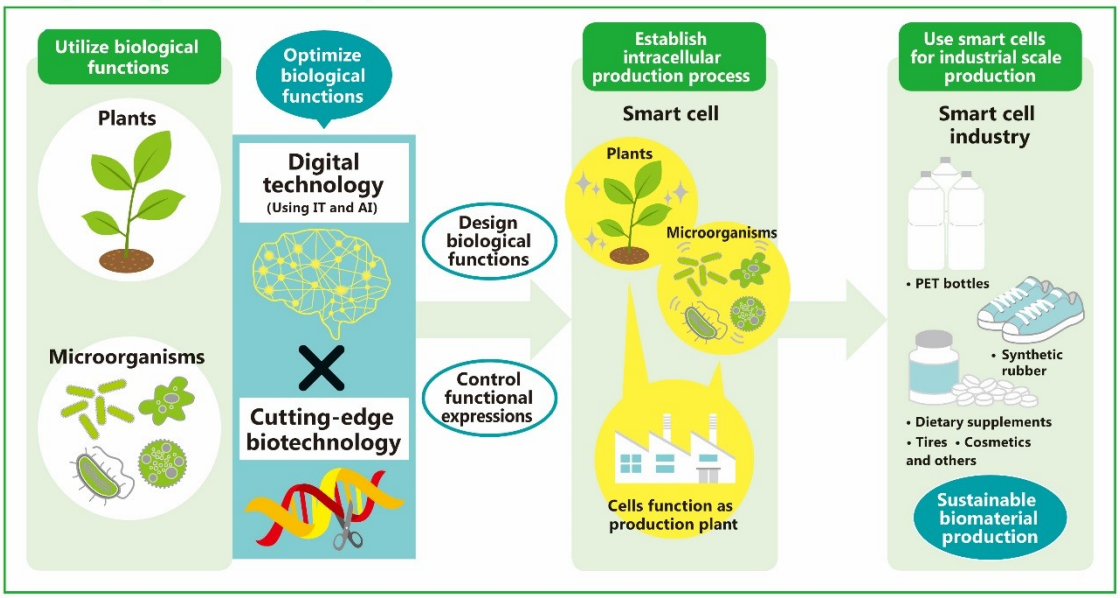
"Project for Development of Production Techniques for Highly Functional Biomaterials Using Smart Cells of Plants and Other Organisms"
Deputy Project Leader : Dr. Takeshi Matsumura
Plant Molecular Technology Research Group Leader,
Bioproduction Research Institute, AIST

Contents

Project Overview

NEDO launched the Smart Cell Project "Project for Development of Production Techniques for Highly Functional Biomaterials Using Smart Cells of Plants and Other Organisms" (FY2016-2020) to create smart cells that are highly optimized for the use in manufacturing process from plants and microorganisms.

Conceptual Diagram of the NEDO Project



Research and development schedule

	FY2016	FY2017	FY2018	FY2019	FY2020	FY2021
① Development of fundamental technologies pertaining to plant productivity control (commission)	Development of domestic genome editing technology Development of gene manifestation control technology for metabolic systems Development of manifestation control technology based on cultivation & growth environments			Verification of suitability to actual plants & technology improvement		
② Development of production techniques for highly functional biomaterials using plants (subsidized)	Identification of metabolic pathways and key genes Development of genetic transformation technology			Optimization of environmental conditions & productivity verification		
③ Development of data analysis system efficacy for high-productivity microbe manufacturing (commission)	Development of high-productivity microbial design system Development of high-throughput synthesis, analysis & assessment methods		Efficacy verification	Development system improvement & package development		
④ Development of production techniques for highly functional biomaterials using microbes (subsidized)				Development of practical targets through system utilization		

Stage gate: Interim assessment

Ex-post assessment

・Activities aimed at commercial operation
・Activities aimed at commercialization of development technology

Project Framework

Implementation Structure (FY2016-FY2020)

NEDO — Advisory board

progress/result report ⇅ advice

Instruction ⇔ Discussion

PL Dr. Satoru Kuhara
(Kyushu Univ. ・ Professor emeritus)
SPL Dr. Takeshi Matsumura
(AIST ・ Plant molecular technology research group leader)

commissioned program

R&D field ①
Development of basic and fundamental technologies for bio-production using plant

(1) Establishment of Japanese technological platform of genome editing
- Tokushima Univ., Meiji Univ., RIKEN, KINDAI Univ.
- Kyushu Univ., Kobe Univ., Hiroshima Univ., The Univ. of Tokyo, AIST, Takasaki Univ. of Health and Welfare, EditForce Inc., Tokyo Medical and Dental Univ. (2016-2018), IPSN (2016-2018)
- AIST, Univ. of Tsukuba

(2) Expression control technology of metabolic gene expression
- Kazusa DNA Research Institute [Recommissioned: Tohoku Univ.]
- Kyoto Univ. [Recommissioned: Amino Up Co., Ltd.(2019-)]
- AIST, Hokkaido Univ., Nara Institute of Science and Technology, Yokohama National Univ.

(3) Gene expression control using artificial environment and cultivation techniques
- Chiba Univ., Northern Advancement Center for Science & Technology (NOASTEC)

R&D field ③
Development of information analysis system for creation of highly productive microorganisms

(1) High throughput synthesis, analysis and evaluation technology
(2) High productive microbial design system
(3) Verification and improvement of validity of the information analysis system

Kobe Univ. [Recommissioned: Okayama Univ., Chiba Univ., Osaka Univ., Keio Univ. (2016-2018)], AIST [Recommissioned: INTEC Inc. (2016-2017)], Kyoto Univ. (2017-) [Recommissioned: National Institute of Health and Nutrition (2020)], RIKEN, The Univ. of Tokyo, Univ. of Tsukuba (2017-), Tohoku Univ. [Recommissioned: Shinshu Univ., Kagoshima Univ.], Kyusyu Univ., Ishikawa Prefectural Univ., Nagaoka Univ. of Technol. [Recommissioned: Kao Corp.], Niigata Univ. of Pharmacy and Applied Life Sciences (NUPALS), NTIE (2017-), Shimadzu Corp., NIKON INSTECH CO.,LTD (2017-), Ajinomoto Co. Inc., Ezaki Glico Co., Ltd. [Recommissioned: Biojet Company LTD.], KNC Laboratories Co. Ltd., Fuji Oil Holdings Inc., Mitsubishi Chemical Corp., JSR Corp., RITE, Hitachi, Ltd. (2017-), JBA, Nihon Techno Service Co.,Ltd. (2016-2018), Precision System Science Co., Ltd. (2016-2018), Spiber Inc. (2017-2018)

subsidy program

R&D field ②
Development of high-performance product production technology using plants

- Takenaka Corp. [Joint implementation: Health Sciences Univ. of Hokkaido, Osaka Prefecture Univ.]
- Kirin Holdings Co. Ltd.[Joint implementation: Osaka Univ.]
- KNC Laboratories Co. Ltd.[Joint implementation: Kobe Univ.]
- Ajinomoto Co. Inc.[Joint implementation: Kyoto Univ., Tamagawa Univ., Tokushima Univ. (2019-), NOASTEC (2019-), Chiba Univ.(2016-2018)]
- Hokusan Co. Ltd.[Joint implementation: AIST]
- Hokkaido Mitsui Chemicals, Inc.[Joint implementation: Kyoto Univ.]
- Amino Up Co., Ltd. [Joint Implementation: AIST, NOASTEC, Tokushima Univ. (2019-), Hokkaido Univ. (2019)]

R&D field ④
Development of high-performance product production technology using microorganisms (2019-2020)

- Toray Industries, Inc. [Joint implementation: AIST, RIKEN]
- Asahi Kasei Pharma Corp. [Joint implementation: AIST]
- NAGASE & CO., LTD. [Joint implementation: AIST, Nara Institute of Science and Technology, Kobe Univ., Tohoku Univ.]
- Fukuoka Soy Sauce Brewing Cooperation [Joint implementation: Kyusyu Univ., Kyoto Univ., AIST, RIKEN]
- Amano Enzyme Inc. [Joint implementation: Kyoto Univ., AIST]

Project Framework

Prior survey

○ Studies on R&D direction in the field of the material production by using Smart Cell (2016／ Mitsubishi Chemical Research Corp.)
○New regulation proposal on genetically modified organism in contained use (2016／ JBA)
○Analysis of the ripple effect on environment and economy in the field of material production by using Smart Cell and the trend survey of the related technologies (2017-2018／ Mitsubishi Chemical Research Corp.)
○Examination of new issues to be solved for promotion of social implementation of smart cell related technology

・Research area: Development of an integrated web tool for novel genome editing systems
(2018-2019／Meiji Univ., Tokushima Univ.)
・Research area: Development of innovative delivery of molecules to genome-edit plants
(2018-2019／BEX CO., LTD. [Recommissioned: Chiba Univ.], Kyusyu Univ.)
・Research area: Research and Study for High Efficiency Methane Production by Applying Deep Learning Analysis to Gene Expressions of Anaerobic Microbial Community and Real Time Analysis of Odorous Components
(2018-2019／Hitachi Zosen Corp. [Recommissioned: Hitz biomass lab inc., Global Center for Medical Engineering and Informatics, Osaka Univ.])
・Research area: Development of robust microorganisms and simple production process
(2018-2019／JGC JAPAN CORPORATION, Sojo University, Nara Institute of Science and Technology)
・Research area: Extraction of tasks for socially implementing isoprene and highly functional isoprene derivative manufacturing technology derived from plant materials
(2018-2019／Mitsubishi Chemical Research Corp., Bridgestone Corp., JSR Corp., Mitsubishi Chemical Corp.)
・Research area: Development of a Platform for Label-Free Sorting of Highly Productive Cells by High-Performance Raman Flow Cytometry
(2018／euglena Co., Ltd., The Univ. of Tokyo)
・Research area: Study on conversion of raw materials and diversification of outlets for C4 chemicals
(2018／Chitose Laboratory Corp., Daicel Corp. [Recommission: Nihon Univ., Tohoku Univ.]
・Research area: Search for Useful Monomer Compounds Synthesized Biologically
(2018／RIKEN, AIST)

○ Benchmark survey for realization of bio-economy society (2019-2020／Mitsubishi Chemical Research Corp.)

Advisory board (2016-2020)

<R&D field ① and ②>

Chairman	Shigeru Kuwata (Meiji Univ.)
Member	Toshio Aoki [-2018] (Nihon Univ.)
Member	Akiko Ishii (National institute of health sciences)
Member	Tetsuya Ishii (Hokkaido Univ.)
Member	Yoshihiro Otaki (Biofrontier partners, Inc.)
Member	Junichi Mineno (Takara Bio Inc.)
Member	Takahiko Yano [2019-] (Taisyo pharmaceutical holdings)

<R&D field ③ and ④>

Chairman	Kuniki Kino (Waseda Univ.)
Member	Yoshihiro Otaki (Biofrontier partners, Inc.)
Member	Katsuya Gomi (Tohoku Univ.)
Member	Minoru Seki (Chiba Univ.)
Member	Hirotada Mori (Nara Institute of Science and Technology)

(names listed) without honorific titles

Chapter 1

Common platform technology

Development of novel genome editing tools using molecular evolution and molecular dynamics approaches:

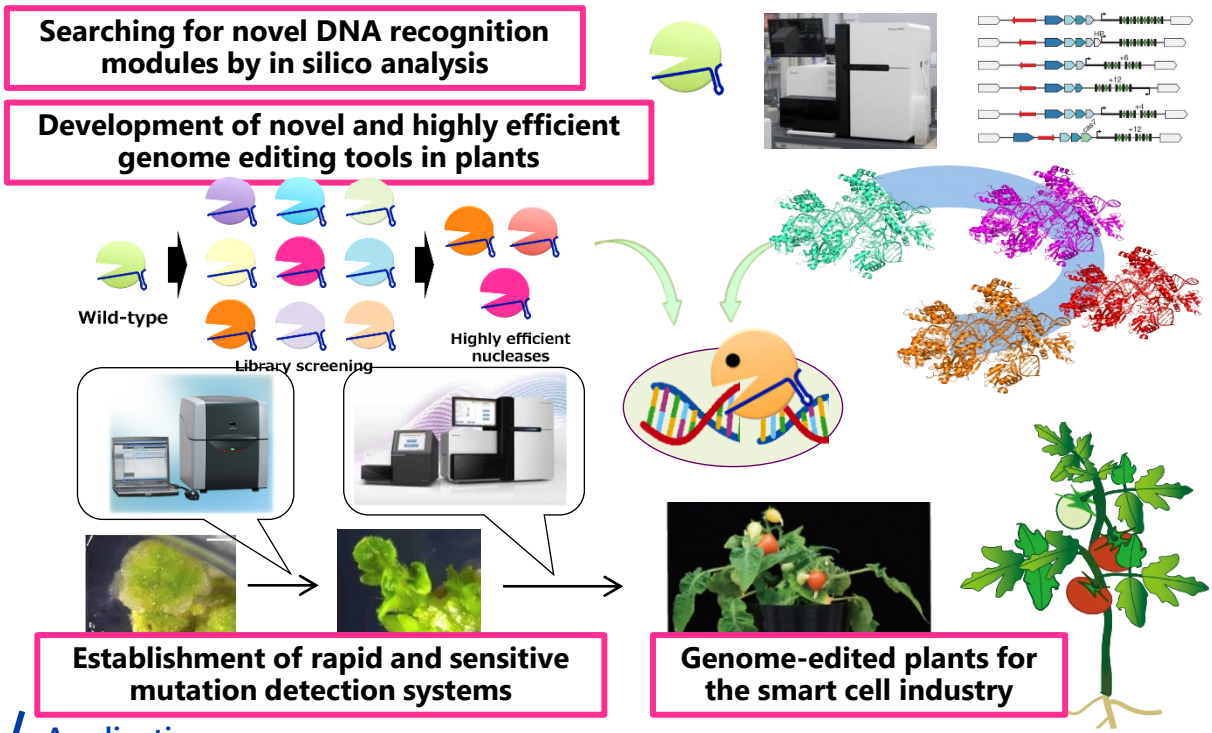
Development of a novel genome editing tool using molecular evolution engineering

Tokushima University

Common Platform Technology

Technical Description

Most of the basic patents for genome editing have been issued by the United States and Europe. Therefore, there is a strong desire to develop novel genome editing tools that can contribute to the activation of the Japanese biotechnological industry. We developed a novel genome editing system, which had not been utilized for genome editing, from metagenome data. This tool could introduce a site-directed mutation on the target of interest in plants.



Applications

- Breeding of metabolically engineered plants and cells for the production of useful chemicals
- Breeding of varieties of agricultural, forestry, and fishery products with useful traits
- Application in the fields of medicine and drug discovery

References

- Patent application No. WO/2019/039417 "Target sequence specific alteration techniques using nucleotide target recognition." (Tokushima University)
- Patent application No. PCT/JP2020/11283 "Targeted sequence modification technology using the CRISPR type I-D system." (Tokushima University)

Contact Information

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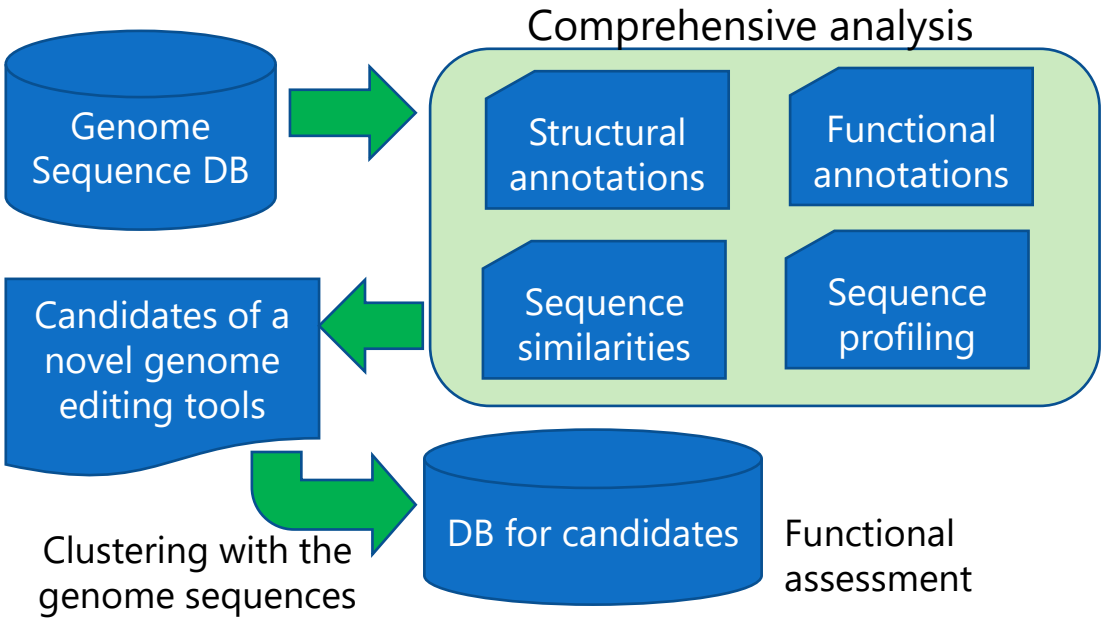
Development of novel genome editing tools using molecular evolution and molecular dynamics approaches:

Large-scale data mining for candidates of novel genome editing tools

Meiji University

Technical Description

We constructed a rapid and high-throughput method for mining candidates of a novel genome editing system from large-scale genome sequence databases. From the genome database, we developed a novel genome editing system, which has not been utilized for genome editing. This tool could introduce a site-directed mutation on the target of interest in plants.



Applications

- Breeding of metabolically engineered plants and cells for the production of useful chemicals
- Breeding of varieties of agricultural, forestry, and fishery products with useful traits
- Application in the fields of medicine and drug discovery

References

Contact Information

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Development of novel genome editing tools using molecular evolution and molecular dynamics approaches:

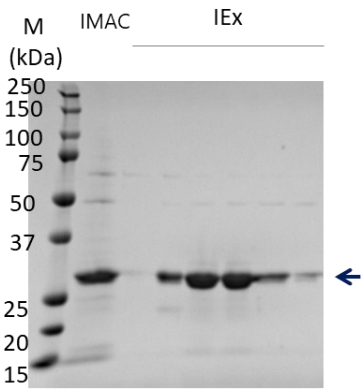
Structural analysis of TiD systems

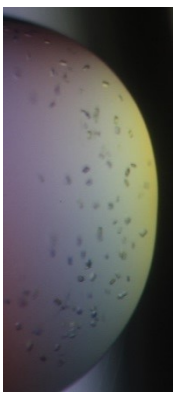
RIKEN

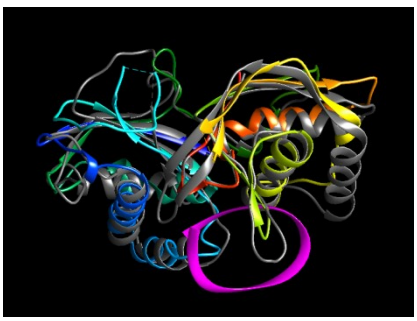
Common Platform Technology

Technical Description

For the utilization and improvement of genome editing tools, the understanding of their molecular structure and the analysis of their working mechanisms are essential. In addition, it requires the expression and purification of large quantities of recombinant proteins that comprise the genome editing tool for structural analyses and experiments in vitro. The expression and purification of recombinant proteins will also serve as a basis for genome editing using purified recombinant proteins as well as for these basic analyses. In this study, we established expression and purification systems using Escherichia coli for each protein subunit of the TiD system and analyzed their crystal structures.







Applications

- Understanding the operating principle of the TiD system based on molecular structure
- Improvement of TiD system based on molecular structure
- Genome editing using recombinant purified proteins

References

Contact Information

RIKEN, BDR
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Development of novel genome editing tools using molecular evolution and molecular dynamics approaches:

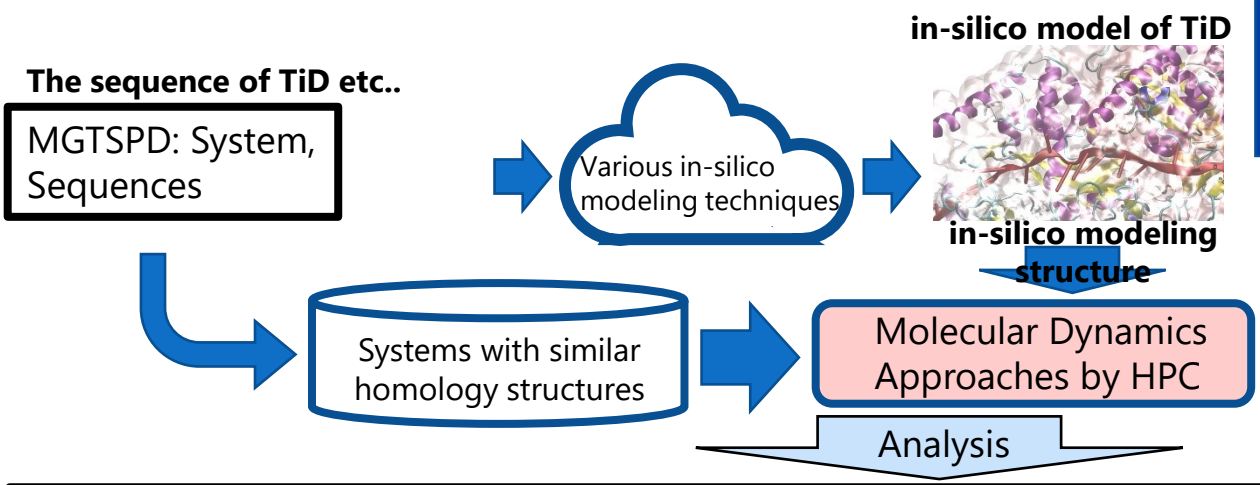
Molecular Dynamics Approaches to support the Development of novel genome editing systems

Kindai University

Common Platform Technology

Technical Description

Our group has several techniques for in-silico modeling of protein/nucleic acid systems and molecular dynamics simulations to support the development of novel genome editing tools. We proposed the modification of TiD and other systems. We also have performed MD simulation of Type I and III systems and so on.
1) To understand the interaction, the dynamics, or the conformation of the system which has not been solved the tertiary structure, we proposed an in-silico model structure of the genome editing system (TiD), the dynamics, and the interaction of the systems.
2) We performed the molecular dynamics simulations of novel genome editing tools and elucidated the mechanism of the interaction and dynamics in the system. To help the development of novel genome editing tools, we proposed the regions for the mutation and the new sequences for Cas7d in TiD and so on.



We proposed the regions of mutation, the new sequence and the dynamics of the system.

Applications

- Developer for novel genome editing tools
- The fields of medicine and drug discovery
- Detail analysis of the function of genome editing systems

References

・Software： Gromacs, MODELLER, ROSETTA, BLAST, ProDy, in-house software etc..

Contact Information

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Transcriptome engineering using designer PPR protein:

In vivo control of translation and splicing

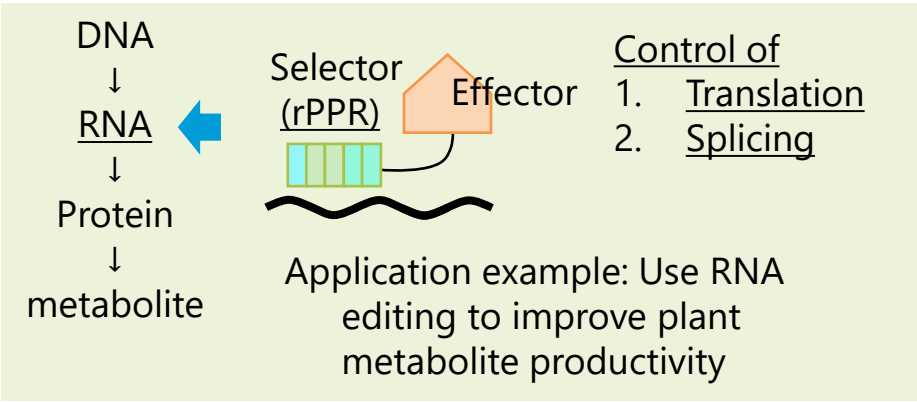
Kyushu University

Technical Description

Summary: It is possible to provide designer RNA binding proteins using PPR motifs and related applications, including splicing and translation, to edit or engineer in vivo RNA without DNA alternation.

Technical advantages :

- PPR the world's first general-purpose RNA engineering technique (patent approved)
- Change of genome function at the RNA level with no alternation of genomic DNA
- The technique enables spatiotemporal regulation of essential RNA in vivo



Applications

- Engineering of metabolic pathway for production of valuable materials
- Pharmaceutical use targeting aberrant endogenous RNA
- Diagnosis and therapeutic use against RNA viruses

References

- Designer RNA binding proteins using PPR motifs (WO/2013/058404)
- Translational control using designer PPR proteins (2016-120524)

Contact Information

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Development of multipurpose genome engineering tools:

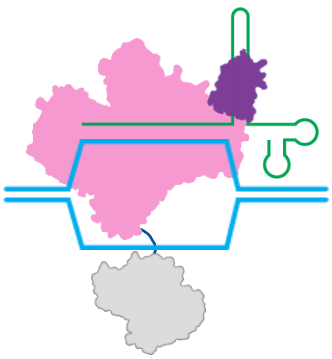
Novel RNA-guide, rational evolution, alternative recombination, organelle transformation

Kobe University

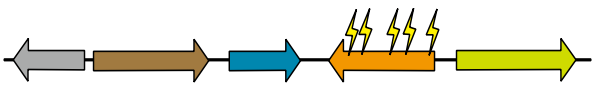
Common Platform Technology

Technical Description

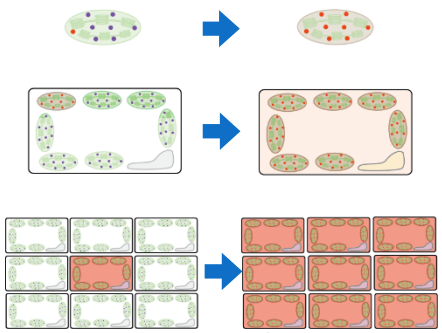
To match the various needs of breeding, more sophisticated genome engineering tools and methods are being developed that include novel RNA-guided genome editing tools, diversification of the specific locus of genomes, stable transformation of plant organelles, and alternative ways of chromosome recombination.



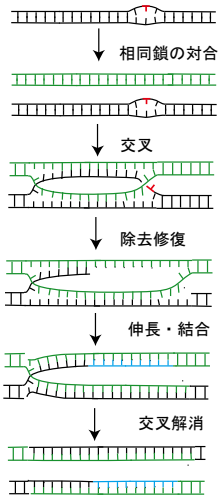
Non-CRISPR type Novel RNA-guided editor



Local genome diversification



Development of new traits using stable transformation of chloroplast and mitochondria



Alternative recombination for chromosome swapping

Applications

- Diversification and screening of useful gene functions
- Genetic manipulation of chloroplasts and mitochondrial genes
- Chromosomal rearrangement

References

- WO2016072399A1: Method for modifying genome sequence to introduce specific mutation to targeted DNA sequence by base-removal reaction, and molecular complex used therein
- WO2019189147: Method for modifying double-stranded DNA sequences in cells
- PCT/JP2020/009553: Method for identifying organelle promoters and their sequences
- JP2020-054225: Method for selection of transformed organelles and its selection marker

Contact Information

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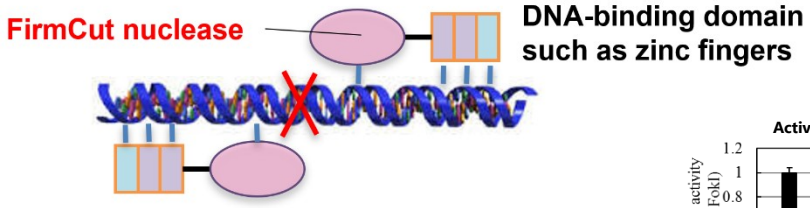
Improvement of DNA cleavage domain and development of single-base-pair editing technology

Hiroshima University

Common Platform Technology

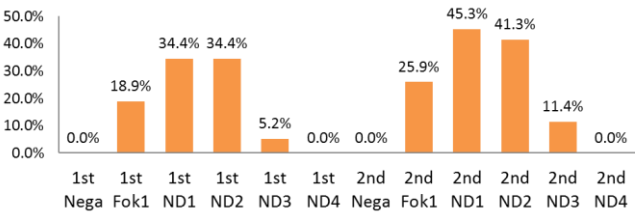
Technical Description

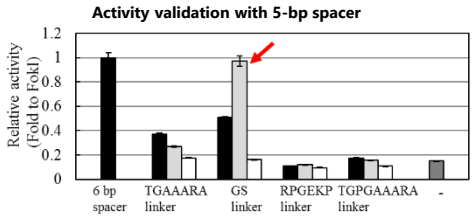
We developed **"FirmCut nuclease"** (patent applied for), a novel nuclease possessing higher functionality such as higher cleavage activity and higher flexibility to various target sequences compared to FokI, which is commonly used for the DNA cleavage domain.

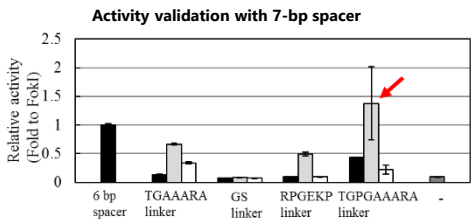


High flexibility (right) and high cleavage activity (bottom) of FirmCut nuclease

ND1, one of the FirmCut nucleases, retains its activity even when the spacer lengths are 5 or 7 bp (right, red arrows).







Enhancement of genome editing activity by ND1 and ND2 (left).

Applications

- Enhancement of the functionality of FokI-based genome editing tools such as Platinum TALEN
- Gene modification using protein-based tools
- Application to made-in-Japan genome editing modules such as PPR

References

- Polypeptides containing DNA-binding domains（2015-33365）
- Novel nuclease domains and uses thereof（PCT/JP2019/033045）

Contact Information

Takashi Yamamoto, Hiroshima University
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Precise genome engineering using recombinase:

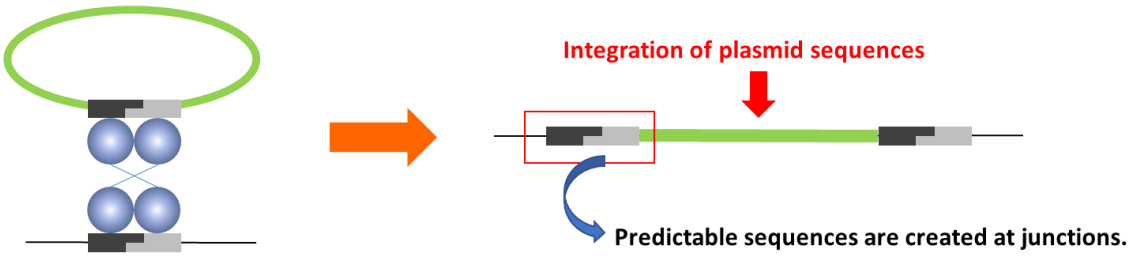
Synthetic recombinases for genome editing

Hiroshima University

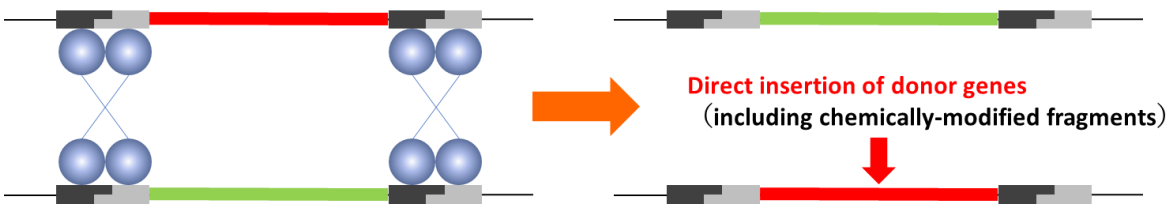
Common Platform Technology

Technical Description

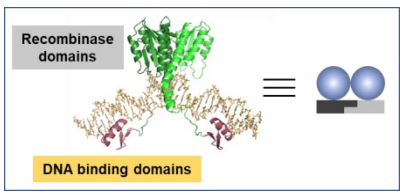
Site-directed insertion of plasmid or large gene fragments



Direct sequence-substitution by cassette exchange



① DNA bindig units are exchangeable
② Precise DNA recognition/recombination via tetramer formation
③ Precise integration or recombination is not easy by CRISPR-Cas gene editing



Applications

- When needed for large gene fragment insertion, which is not efficient by nuclease-based gene editing
- When needed for alternate integration methods such as Flp-in® with expanded site options

References

- Nomura, W., et al. "Effects of DNA Binding of Zinc Finger and Linkers for Domain Fusion on Catalytic Activity of Sequence-Specific Chimeric Recombinases Determined by a Facile Fluorescent System." *Biochemistry* 51; 1510-1517, 2012.

Contact Information

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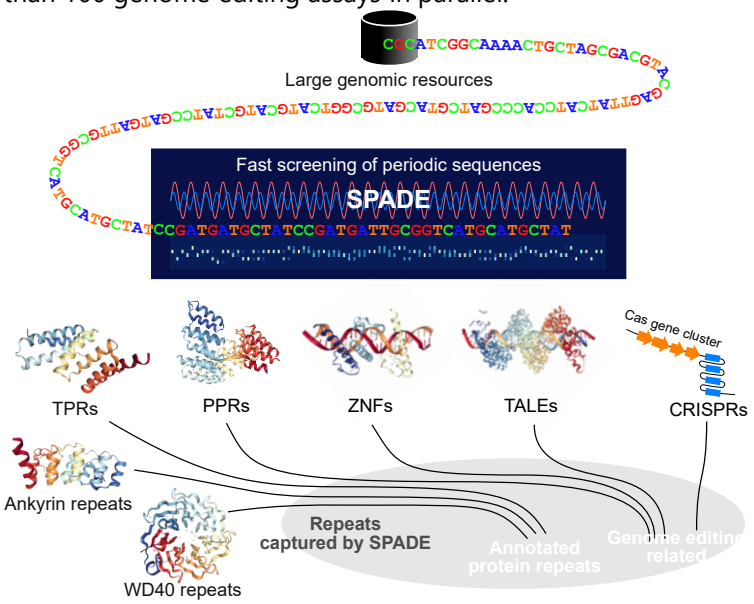
Computational platform for genome editing: Exploration and evaluation of novel genome editing tools

The University of Tokyo

Common Platform Technology

Technical Description

We have developed (1) SPADE, a universal software to rapidly explore novel genome editing modules, and (2) the DIAMOND system, a high-throughput platform to evaluate new genome editing technologies. We demonstrated that SPADE successfully extracted a range of reported genome editing modules from genomic resources with a performance that was on par or higher than other specialized software tools. DIAMOND enabled analyzing genome editing outcome patterns of more than 100 genome editing assays in parallel.



Applications

- Development of novel genome editing tools
- Agriculture, bioproduction, genome editing therapeutics

References

- Mori H, Evans-Yamamoto D, Ishiguro S, Tomita M & Yachie N. Fast and global detection of periodic sequence repeats in large genomic resources. (2019) Nucleic Acids Research 47, e8
- Sakata RC, Ishiguro S, Mori H, Tanaka M, Tatsuno K, Ueda H, Yamamoto S, Seki M, Masuyama N, Nishida K, Nishimasu H, Arakawa K, Kondo A, Nureki O, Tomita M, Aburatani H & Yachie N. Base editors for simultaneous introduction of C-to-T and A-to-G mutations. (2020) Nature Biotechnology 38, 865-869

Contact Information

Research Center for Advanced Science and Technology, The University of Tokyo
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Delivery technology for genome editing: Protein delivery into plants

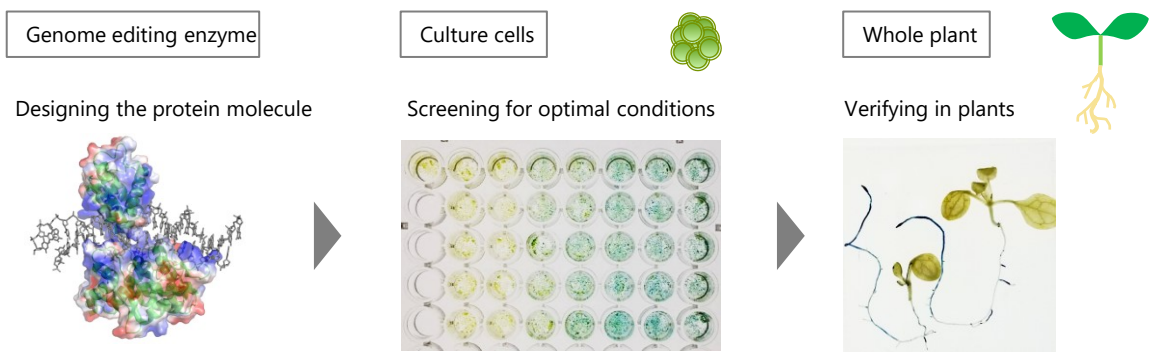
National Institute of Advanced Industrial Science and Technology (AIST)

Technical Description

Protein internalizations into cells play important roles for biological functions in various organisms. By designing the protein architecture, proteins for genome editing are delivered into plant cells.

Technical advantages:

- Delivery of biomacromolecules in plant cells with intact cell walls
- Genome editing without delivering nucleic acid materials



Applications

- Delivery of genome editing modules into plant cells
- Creating genome editing plants without any insertion of foreign genes

References

- Furuhata Y., et al., *Sci. Rep.* 9, 2163 (2019)
- Furuhata Y., et al., *Plos One*, 15, e0227477 (2019)

Contact Information

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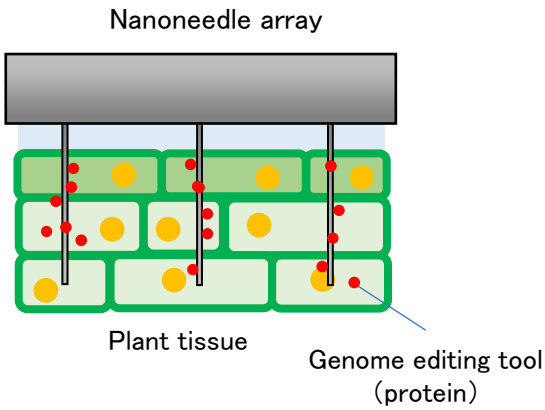
Delivery of genome editing modules into individual plants using nanoneedles: Molecular delivery by mechanoporation

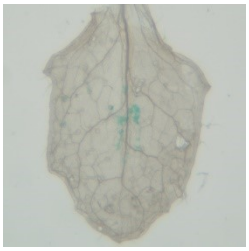
National Institute of Advanced Industrial Science and Technology (AIST)

Technical Description

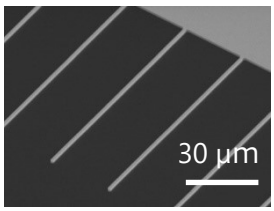
Summary
We developed a technique for genome editing by physical insertion and direct material delivery into plant tissue, including stem apical meristematic tissue, using nanoneedle arrays of ultra-fine needles with a high aspect ratio.



Leaf tissue stained with genome editing



Nanoneedle array



Molecular delivery using nanoneedles
We succeeded in genome editing by direct delivery of protein tools to Arabidopsis tissue using nanoneedle arrays.

Applications

- Any plant species for which the host vector system has not yet been developed
- It can be manipulated against microtissues such as stem apical meristematic tissue

References

- JP2020-053154 “Microneedle arrays and a method for introducing substances into plant cells using arrays”
(filed March 24, 2020; unpublished)

Contact Information

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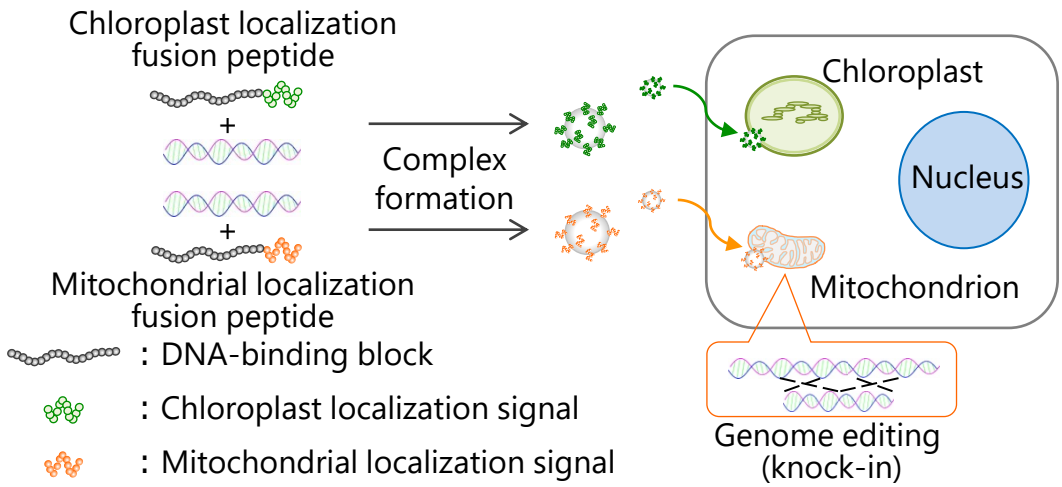
Organellar genome editing technology:

Organellar genome editing (gene knock-in) using organellar localizing fusion peptides

Takasaki University of Health and Welfare

Common Platform Technology

Technical Description



Plant cells have two organelles such as chloroplasts and mitochondria which show an advantage in bioproduction of industrial and medical substances. We are developing selective organellar genome editing technology using organellar localization fusion peptides. Using this technology, exogenous DNA is able to be delivered only into target organelles. Additionally, this is the only technology of mitochondrial genome editing in plants.

*Since homologous recombination occurs frequently in chloroplasts and mitochondria, genome editing (knock-in) is possible if a foreign gene with a homologous sequence can be delivered.

Applications

- Seed companies
- Agricultural biotechnology companies

References

- Yoshizumi *et al.*, (2018) Selective Gene Delivery for Integrating Exogenous DNA into Plastid and Mitochondrial Genomes Using Peptide-DNA Complexes. ***Biomacromolecules*** 19: 1582-1591
- Kimura *et al.*, (2019) A centrifugation-assisted peptide-mediated gene transfer method for high-throughput analyses. ***Plant Biotechnol.*** 36: 49 - 52

Contact Information

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Development of DNA binding modules:

Establishment of technology for genome editing using DNA-binding PPR proteins

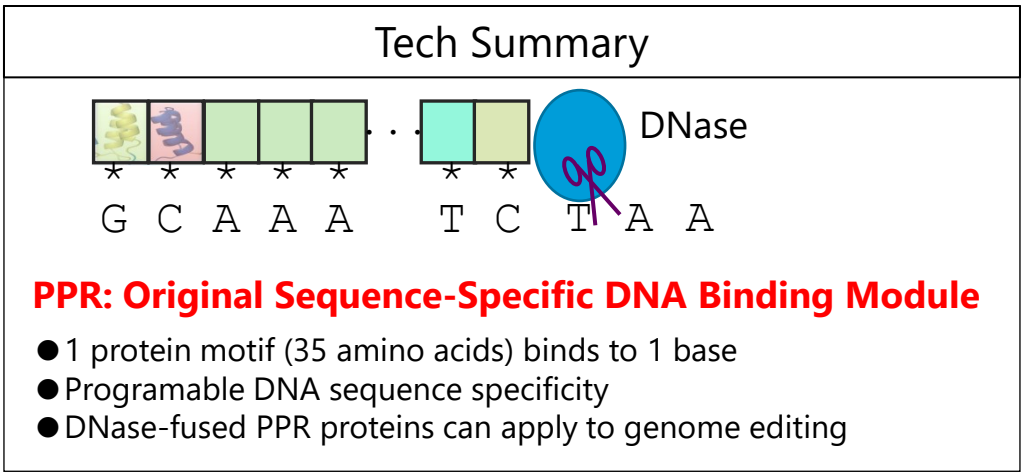
EditForce, Inc.

Technical Description

Summary: We have an original design method for sequence-specific DNA binding proteins using PPR proteins. This can provide a molecule that can be used to knock out any target gene (genome editing).

Technical advantages:

- DNA binding modules that are completely different from ZincFinger, TALE and CRISPR systems
- Unique domestic technology with basic patents



Applications

- Modification of secondary metabolite production pathways
- Creation of genome-edited organisms of genes that cannot be targeted by existing methods

References

- DNA BINDING PROTEINS USING PPR MOTIF, AND USE THEREOF （WO2014175284A1）

Contact Information

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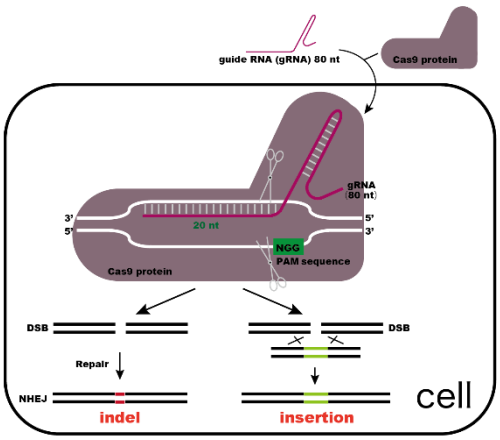
Development of a novel genome editing method from Japan: Confirmation of PODiR system in plants

National Institute of Advanced Industrial Science and Technology,
University of Tsukuba

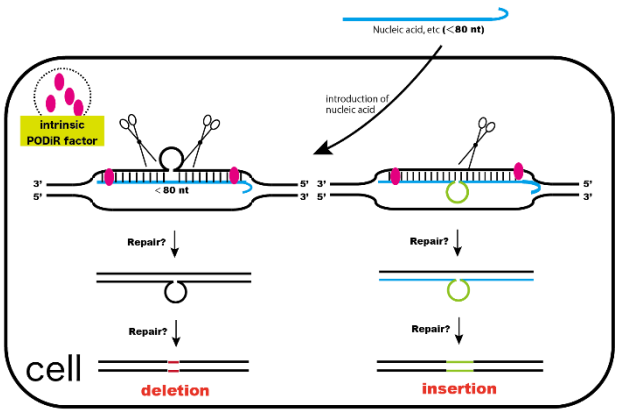
Technical Description

1. What is the PODiR system?

The PODiR system is one of the bacterial antibiotic resistance acquisition systems, and its presence is expected not only in bacteria but also in animal cells and plant cells. By applying this system, genome editing different from CRISPR-Cas9 can be expected.



CRISPR-Cas9



PODiR system

2. PODiR system for plants (cells)

Assay cells are created and implemented to confirm that the PODiR system works in microorganisms, animals (cells), plants (cells), etc.

Applications

- General breeding of living cells and things
- Implementation of precise gene therapy
- Creation of highly functional cells for bioenergy production

References

- Tokugan2015-111458 Expression cassettes for eukaryotic cells that enable a new expression induction system
- Tokugan2015-149826 Protein expression methods
- Tokugan2018-533576 Genome editing methods

Contact Information

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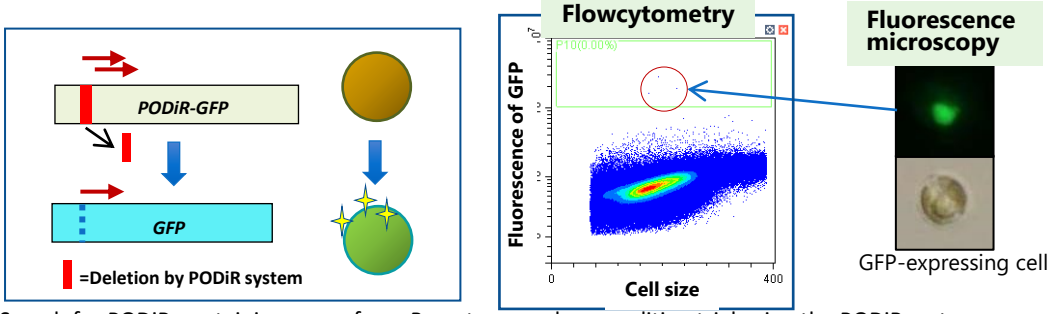
Development of a novel genome editing method from Japan:
Confirmation of PODiR system in algal cells

National Institute of Advanced Industrial Science and Technology
University of Tsukuba

Common Platform Technology

Technical Description

1. Confirmation of a PODiR system in a haptophyte alga, *Pleurochrysis carterae*
→We synthesized a PODiR (Partly Overlapped Direct Repeat)-containing GFP gene (*PODiR-GFP*) which encodes a non-fluorescent GFP, and transformed the *P. carterae* with that modified gene. Functional activation of a spontaneous PODiR system will be detected by the fluorescence of the GFP.



2. Search for PODiR-containing genes from *P. carterae* and gene editing trial using the PODiR system
→We identified approximately 2,500 PODiR-containing genes from the mRNA database of *P. carterae*. As described below, PODiR sequences were conserved in various genes.

Examples of PODiR-containing genes

Metabolism: UDP glycosyltransferase, Glyceraldehyde-3-phosphate dehydrogenase, etc.
Cell Cycle: Cyclin, etc.
Photoreception: Blue light receptors, Fucoxanthin chlorophyll *a/c*-binding proteins, etc.
Cytoskeleton: Tublin, Cytoplasmic dynein, etc.

→We are now trying to edit a Lipase gene to obtain mutant cell lines with high lipid storage.

Applications

Establishment of mutant cell lines with high lipid accumulation or lipid synthesis activity

References

A genome editing system via ssDNA (single-stranded DNA) (Patent Pending)

A novel constitutive gene suppression system (Patent Pending)

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Establishment of a new genome editing platform

Kyushu Univ., Tokushima Univ., Kobe Univ., Hiroshima Univ., Tokyo Univ., Tsukuba Univ., AIST, RIKEN, Takasaki Univ. Health & Welfare, Kinki Univ., Meiji Univ., EditForce Inc.

Technical Description

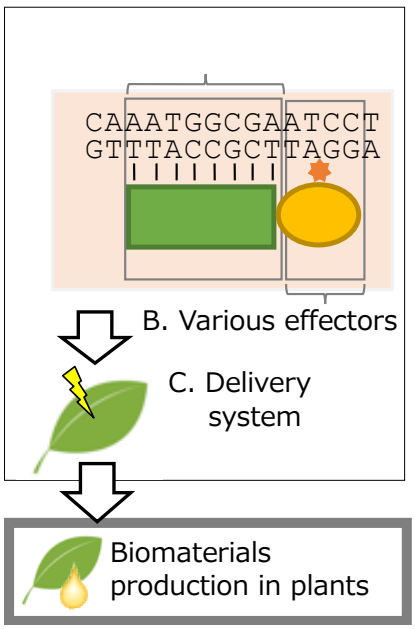
The R&D is focusing on a series of genome editing techniques including (A) DNA recognition modules, (B) various effectors, (C) delivery systems, (D) support units, (E) IP strategies, and further, the packaging of them to establish user-friendly applications.

A. DNA recognition modules
A-1. DNA-binding PPR (protein-based)
(EditForce Inc)
A-2. TiD (guide RNA-based)
(Tokushima Univ.)
A-3. Novel protein-based module
(Tokyo Univ. et al.)
A-4. PODiR (guide RNA-based; AIST)

B. Effectors and applications
B-1. Various applications (Kobe Univ.)
B-2. Precise genome design using recombinase
(Hiroshima Univ.)
B-3. Novel nuclease (Hiroshima Univ.)
B-4. Organelle genome editing
(Takasaki Univ. H&W)
B-5. Transcriptome editing (Kyushu Univ.)

C. Delivery systems
C-1. DIVE (surface charge control; AIST)
C-2. Nanoneedle (AIST)
C-3. Cell-penetrating peptide
(Takasaki Univ. H&W)

Packaging
1. [DNA-binding PPR]+[novel nuclease]+[nanoneedle/peptide]
2. [TiD]+[nanoneedle/peptide]



Applications

Medical, chemical, agricultural fields

References

Genome Editing Industrial Network
(http://www.mls.sci.hiroshima-u.ac.jp/smg/GEIN/en/index.html)

Contact Information

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Gene-expression ON/OFF switching platform in plants: Regulation of isoprenoid biosynthetic pathways

Kazusa DNA Research Institute / Tohoku University

Common Platform Technology

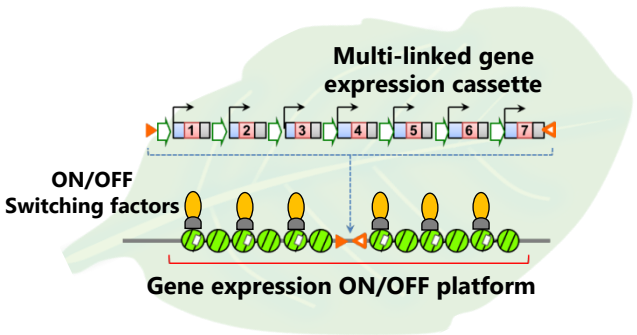
Technical Description

Issues regarding the production of useful materials by plant metabolic engineering

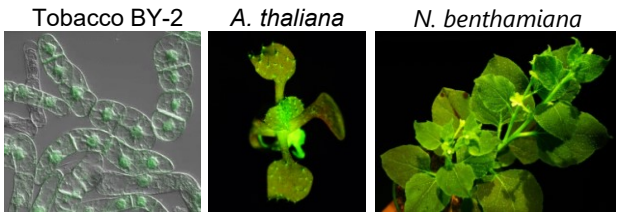
・Introducing multiple genes **requires time and effort**.
・It is **difficult to achieve uniform expression levels** of multiple transgenes with different chromosomal sites.
・Transgene expression is often **suppressed in progeny**.
・**High accumulation of target compounds inhibits the growth** of host plants.

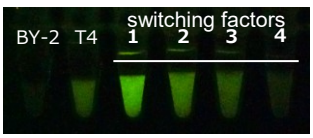
Development of gene expression ON/OFF switching platform using chromatin manipulation

・Introduction of **multi-linked gene cassettes** to specific sites
・**Strict gene expression control** of the site using **chromosome engineering technology**



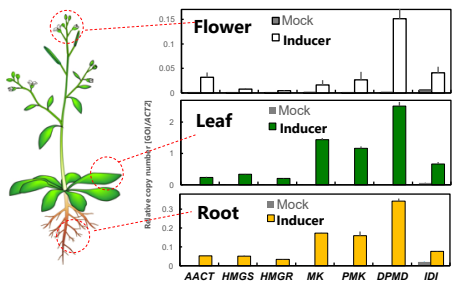
EYFP gene expression from the switching platform





Gene expression control on the platform by switching factors (BY-2 T4 cell line)

Metabolic engineering of isoprenoid biosynthetic pathways



The platform enables us to induce 7 genes in various tissues by the inducer treatment.

Applications

- Construction of a high production platform for plant isoprenoids that has a major impact on industry
- Applicable to a wide variety of genes and useful natural compounds (natural rubber, pharmaceuticals, biofuels, etc.)

References

- PCT/JP2019/030783 (WO2020/031985 A1) Tokugan 2018-152008

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R&D achievements of NEDO smart cell project.

Product-accumulating technology in plants:

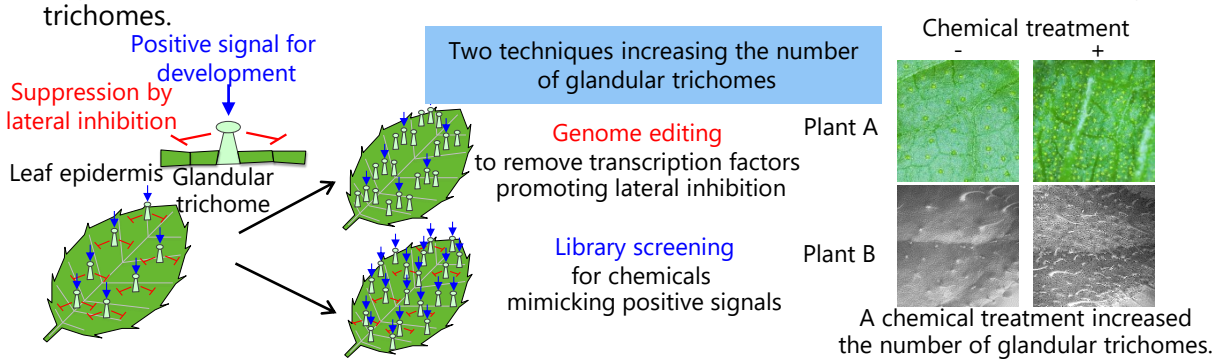
Regulation of glandular trichome differentiation and transport machinery

Kyoto University, Amino Up Co., Ltd.

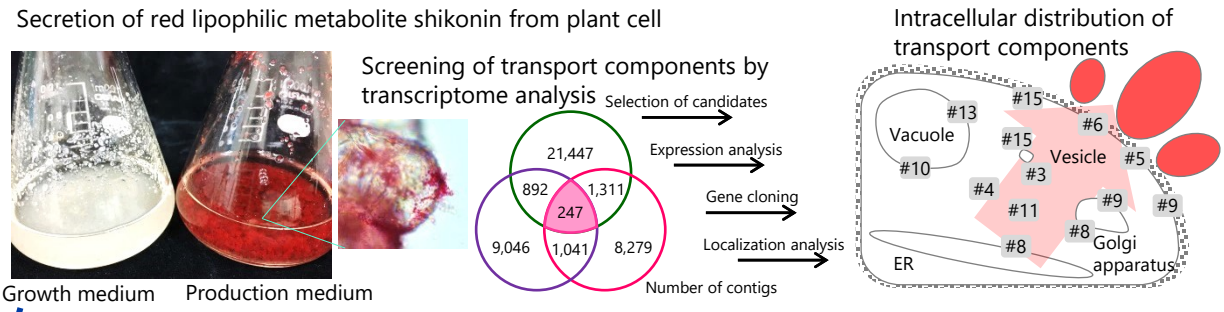
Common Platform Technology

Technical Description

1) Promotion of glandular trichome differentiation: Plants produce functional materials frequently in glandular trichomes. We have developed two techniques that increase the number of glandular trichomes.



2) Dissection and application of transport machinery: Valuable lipophilic metabolites produced by plant cells, e.g. paclitaxel, artemisinin, and limonene, are accumulated in apoplastic spaces. To improve productivity, we are developing a technique that regulates transport activity of lipophilic metabolites.



Applications

- Production of valuable natural products accumulated in glandular trichomes (terpenoids, prenylated flavonoids, etc.)
- Production of lipophilic pharmaceutical intermediates by plant cells
- Production of usable materials by plant cells

References

- Patent Application No. 2018-121203 “Container for high-throughput plant screening tests”
- Tatsumi, K., et al., Plant Biotech., 37 (1): 39-46 (2020). DOI: 10.5511/plantbiotechnology.19.1212a
- Ueoka, H., et al., Plant Physiol., 182 (4): 1933-1945 (2020). DOI: 10.1104/pp.19.00999
- Izuishi, Y., et al., Sci. Rep., 10 (1): Article 13555 (2020). DOI: 10.1038/s41598-020-70469-1

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Development of production techniques for highly functional biomaterials using smart cells of plants and other organisms:

Development of technology for the regulation of DNA methylation of plant endogenous genes using CMV vectors and chromatin remodeling

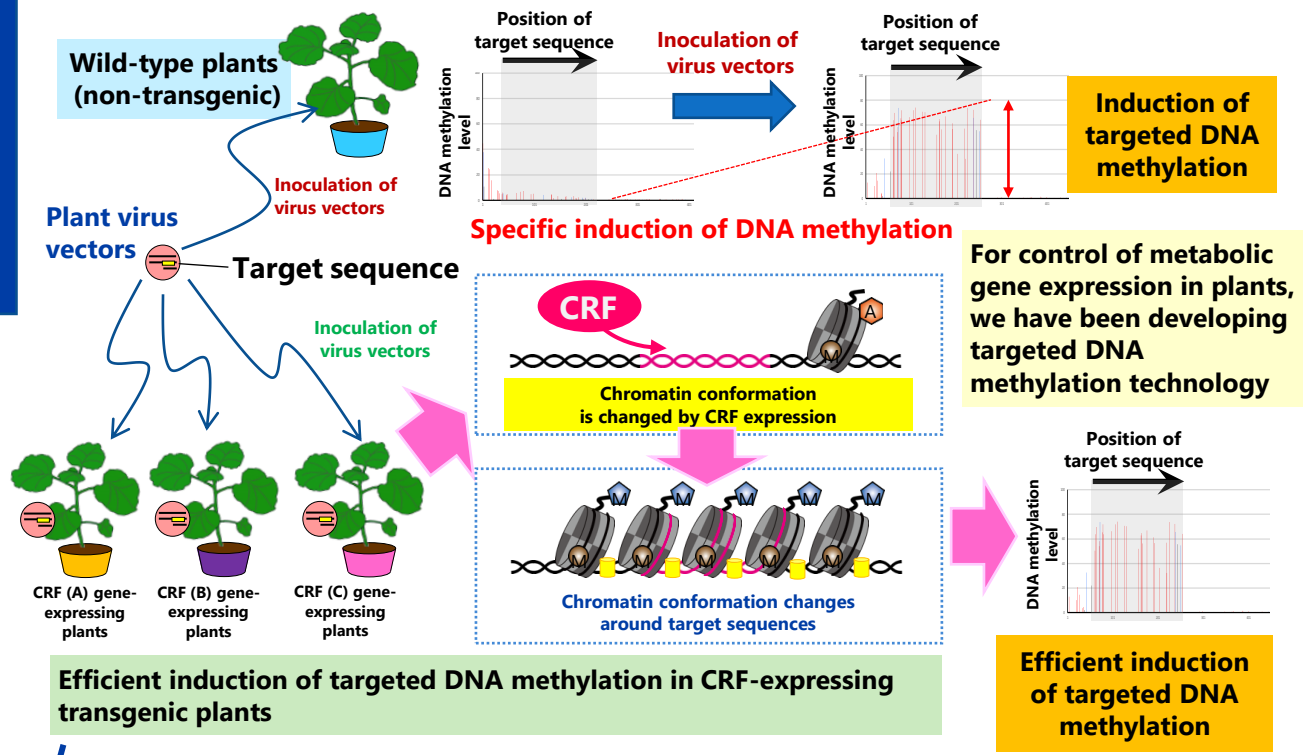
National Institute of Advanced Industrial Science and Technology （AIST）

Common Platform Technology

Technical Description

We have been developing targeted DNA methylation technology enabling the transcriptional repression of desired endogenous metabolic genes for efficient production of useful metabolites in plants. Our patented plant virus vector, cucumber mosaic virus vector could efficiently induce targeted DNA methylation in endogenous genes (virus-induced transcriptional gene silencing, VITGS). We have been also developing technology to induce VITGS more efficiently by changing chromatin structure using transgenic plants expressing chromatin remodelling factor (CRF).

Induction of targeted DNA methylation using plant virus vectors



Efficient induction of targeted DNA methylation in CRF-expressing transgenic plants

Applications

- Production of chemical products, especially plant-derived secondary metabolites
- Production of pharmaceutical and industrial raw materials (recombinant proteins)
- Plant-related research

References

- https://unit.aist.go.jp/bpri/bpri-pmt/result.html

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Regulation of gene expression by sequence-specific demethylation:

A technique to control epigenetics using viral vectors

Hokkaido University

Common Platform Technology

Technical Description

Technical development details

- This study is a sequence-specific induction of gene demethylation in plants.
- Gene expression is controlled by RNA-directed DNA methylation (RdDM) (Fig. 1-①).
- Ribozymes (Rz) expressing from the cucumber mosaic virus vectors (Fig. 1-②) cleave scaffold RNAs or P4-RNA (Fig.1-③) in a sequence-specific manner.

Results

- A ribozyme was designed to target a transposon (TNT1) as an example of endogenous gene. DNA methylation level decreased by 30% compared to that of the empty vector (A1). The TNT1 expression level increased (Fig.2).
- Two weeks after inoculation, even a novel insertion was found in the *N. benthamiana* genome (Fig.3).

Fig.1 RdDM pathway and RNA cleavage mechanism by ribozyme

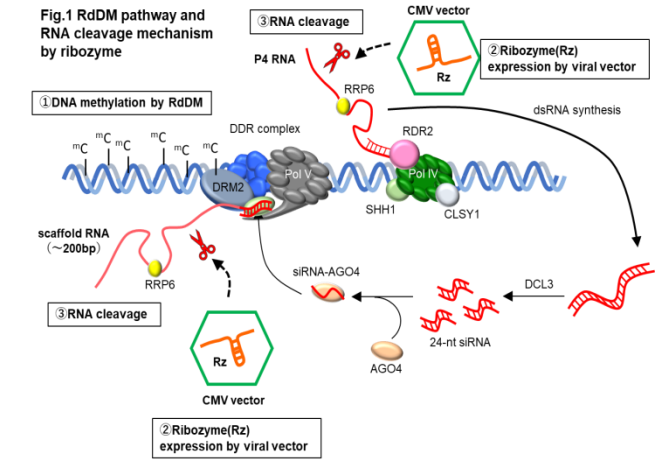


Fig.2 TNT1 expression and DNA methylation level

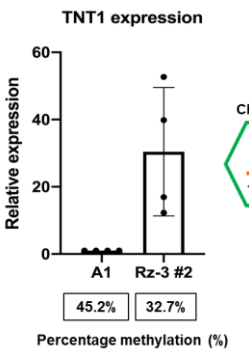
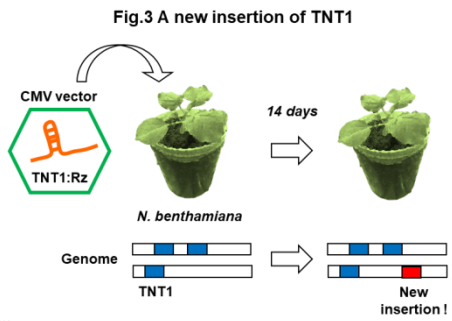


Fig.3 A new insertion of TNT1



Applications

By simply inoculating a viral vector onto plants, we can specifically induce DNA demethylation at the target gene and thus subsequent expression of the gene. Because the methylation level is inherited to the next generation, we can achieve an increase in the level of accumulation of important proteins and secondary metabolites in plants.

References

- Patent: Method for inhibiting methylation of target DNA in plants (Pat. 2018-139316 and Pat. 2019-237374)
- Matsunaga et al., BMC Plant Biology volume 19, 24 (2019)

Contact Information

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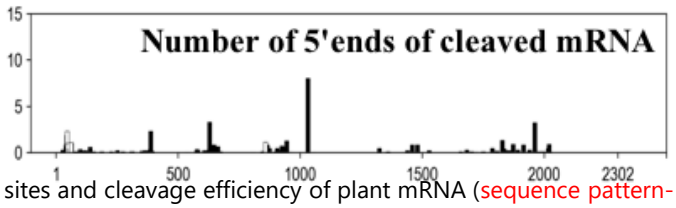
Development of stabilization technology for metabolic pathway related genes

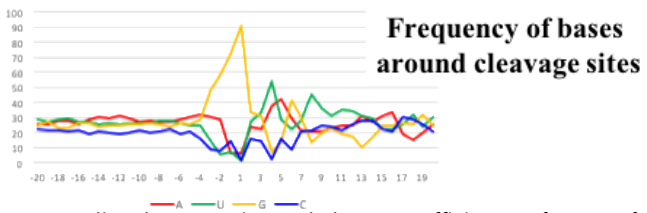
Nara Institute of Science and Technology

Technical Description

*Intracellular mRNA is cleaved internally (one of the factors that does not fully express foreign genes)



*Comprehensive analysis of the cleavage sites and cleavage efficiency of plant mRNA (sequence pattern-dependent cleavage)



*Construction of mathematical model that can predict cleavage site and cleavage efficiency of mRNA from sequence information

*Highly express target gene by modifying mRNA sequence without changing amino acid sequence

Applications

- Efficient expression of foreign genes introduced into plants
  Improvement of plant function by molecular breeding
  Production of useful proteins (including pharmaceutical) using plants (cells)

References

- Ueno, D. et al., *J. Biosci. Bioeng.*, **125** (6), 723-728 (2018)
- Yamasaki, S. et al., *Plant Biotechnology*, **35** (4), 365-373 (2018)
- Ueno, D. et al., *Plant Cell Physiol.* **61** (1), 53-63 (2020)

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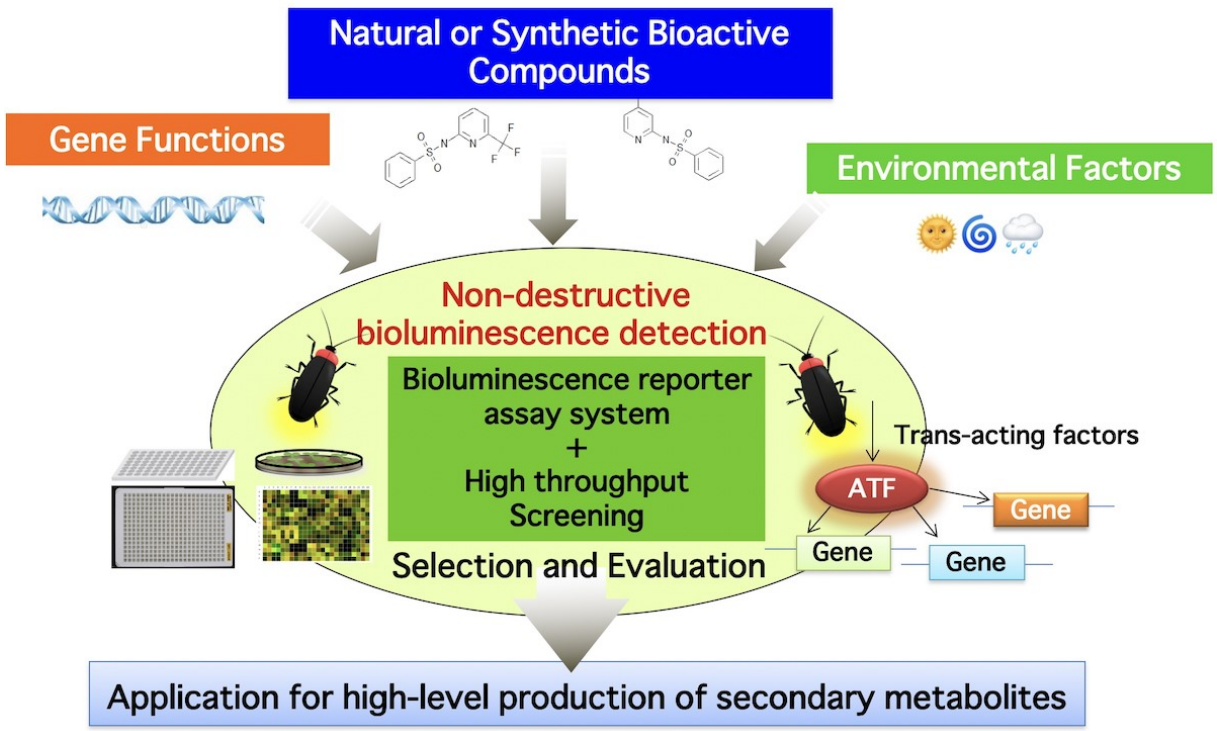
Development of regulated gene expression technologies using factors and regulatory elements: Application of bioluminescence reporter system

Yokohama National University

Technical Description



Applications

- Improvement of ingredient content of medicinal plants using compounds discovered in this study
- Improvement of efficiency of transient expression in plant cells using compounds discovered in this study
- The use of novel transcriptional activators for higher expression levels of targeted genes
- Screening of chemicals that enhance the production of secondary metabolites in plant cells

References

- Patent: Pest control agent, pest control method, transformation efficiency promoter, and transformation efficiency promotion method. P6579539, PCT/JP2015/065242
- Patent: Photo-identification method, substance detection method, reporter assay method, kit, luciferin-luciferase reaction inhibitor, luciferin-luciferase reaction inhibition method and device. P6579539

Contact Information

Kazuyuki Hiratsuka, Yokohama National University
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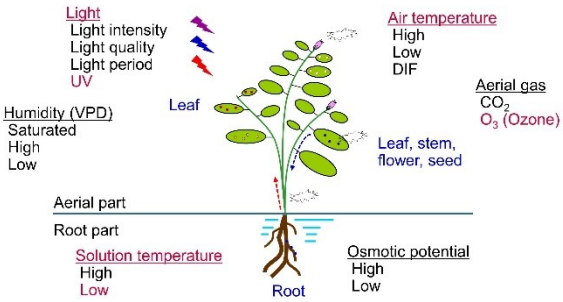
Control of secondary metabolite production through manipulation of plant environments

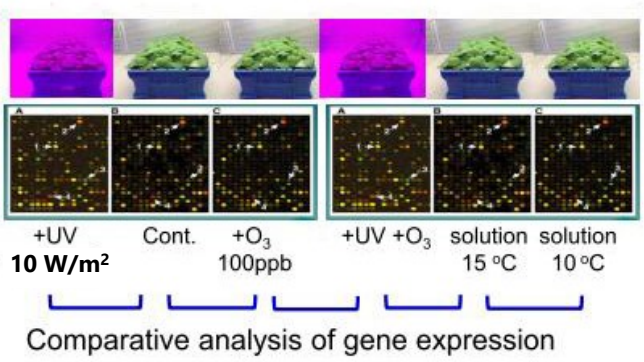
Chiba University

Common Platform Technology

Technical Description

Plants have various abilities to adapt to changes in the environment. This closed-system "plant factory" can provide plants with various artificial stimuli and physiological stresses to maximize accumulation of the target material. In order to assist developing strategies for higher production of secondary metabolites by controlling cultivating conditions, gene expression changes under various cultivating conditions will be examined..





Brassica napus
セイヨウアブラナ

N. benthamiana
ベンサミアーナ

1. Add single or combined environmental stress factors (light, gas, temperature, water stress) to local organ or whole plant.

2. Find key genes responding to the stress factors using gene expression analysis.

3. Analyze gene expression quantitatively and measure target secondary metabolites.

Applications

- The production of poor productive plant-based materials utilized for medicines, health foods, supplements, etc.

Contact Information

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Efficient metabolite production utilizing metabolite-related gene expression profiles: Highly efficient production of metabolites in a controlled environment

Northern Advancement Center for Science & Technology

Technical Description

● Efficient secondary metabolite production technology utilizing controlled environmental stress for a poor productive plant-based material.

We collect numerous expression profiles of genes in the secondary metabolite pathways from plants under different environmental stress conditions and call it "INDEX".
This INDEX presents you some useful factors: some enhance activation of the metabolic pathway to the target, others repress the branching steps to others. We believe that the established INDEX increases efficiency of R & D for high secondary metabolite production.

Target Genes	chemical A	chemical B	ligtht A	ligtht B	…
gene A	→	↗↗↗	↗↗↗	→	…
gene B	↗	↗↗↗	→	↘↘↘	…
gene C	→	↘↘	↗↗	→	…
gene D	→	→	↘↘↘	↗↗	…
gene E	→	→	↗↗	→	…
gene F	→	→	↘↘	↘↘	…
gene G	↘	↗↗	→	→	…
gene H	↗↗↗	↘	↗↗	↘	…
…	…	…	…	…	…

↗: enhance ↘: repress →: no change

Fig. 2 The image of our "INDEX"

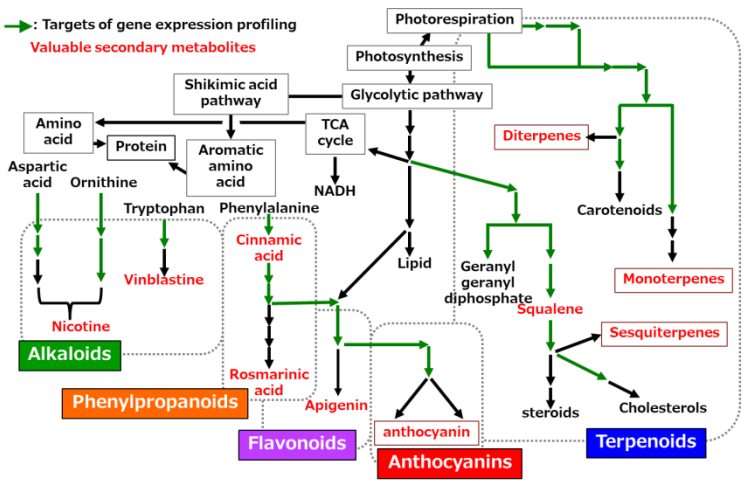

Fig. 1 The main Biosynthesis pathway of plant secondary metabolites

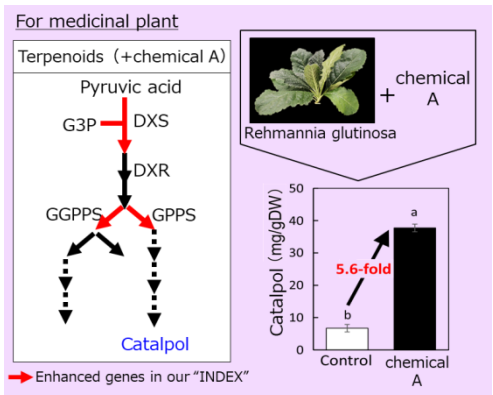

Fig. 3 The example using our "INDEX"

Applications

- Production of a poor productive plant-based materials utilized for medicines, health foods, supplements, etc.

References

- Effects of chemical treatments on expression of genes involved in secondary metabolites of *Nicotiana benthamiana* (IPMB, 2018)

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DBTL overview:
Development of an ultra high-speed smart cell creation platform centered on information technology

Technical Description

We have combined information science and synthetic biological approaches to raise the productivity of substances to a high level in terms of conventional issues such as production of new compounds that could not be produced by conventional microorganisms and enhancement of microbial productivity. We are building a "smart cell creation platform" to speed up problem solving.

Bio-foundry pilot infrastructure (Kobe University)

The DBTL (Design-Build-Test-Learn) cycle is adopted as the basic concept of the platform. In the "Design" area, we are developing a smart cell design system incorporating an information analysis system for metabolic pathway design, enzyme selection and modification, and gene expression control. In the "Build" area, to realize the designed smart cell, we are developing technologies such as long-chain DNA synthesis and high-throughput automatic recombinant construction. The "Test" area is where productivity analysis and various omics analyzes are performed on the microorganisms. The obtained data will be used for feature extraction, which is a technology in the "Learn" area, and that information will be used again for "Design." By rotating this DBTL cycle, microbial breeding is made efficient and smart cells are created.
The platform is applicable to industrial microorganisms. In order to build a consistent smart cell creation platform for the DBTL cycle, we are conducting verification tasks to apply specific substances that companies target. By feeding back the verification results and various data, we are contributing to the sophistication of this platform and developing practical technology while demonstrating its effectiveness.
The next-generation industry that produces high-performance products using smart cells is called the "smart cell industry", and is expected to expand into the industrial fields, agricultural fields, and medical/healthcare fields in the future.

References

- Nature Focal Point
- Japan Bioindustry Association website

Contact Information

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Overview of informatics system:

Computational approaches for smart cells

AIST, RIKEN, Kyoto University

Technical Description

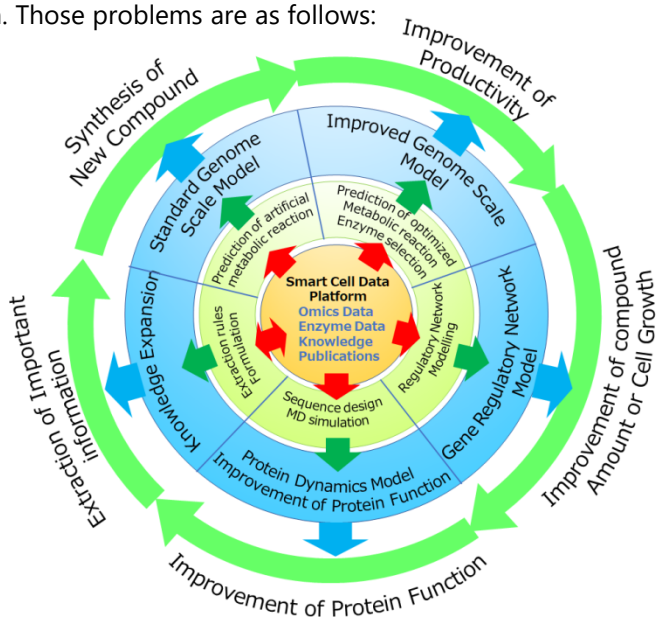
The improvement of microbial production ability is the one of the important themes in the field of bioproduction. In this project, we developed a comprehensive computational system to solve some difficult problems for microbial bioproduction. Those problems are as follows:

1. Synthesis of new compounds
2. Improvement of microbial productivity
3. Improvement of compound amount or cell growth
4. Improvement of protein function

To solve those problems, we have to prepare a data management system. Thus, we have two additional problems:

5. Extraction of important information from knowledge
6. Construction of data management system

In this project, we developed and improved original computational approaches to solve those problems, and the efficiency of the developed approaches were evaluated using empirical methods. By using these methods and this management system comprehensively, we can suggest new modification candidates.



In this figure, the circle at center indicates the data management system. The red arrows indicate data exchange from the system to each approach. The light green circle indicates developed computational approaches, and the blue circle indicates the models constructed using those approaches. The outermost green circle indicates the problems to solve.

Applications

- Improvement of microbial productivity in the bioproduction field

References

- Araki, M., *et al.*, *Bioinformatics*, 31(6), 905-911, 2015
- Shirai, T., *et al.*, *Microbial Cell Factories*, 15(13), 1-6, 2016
- Aburatani, S., *Gene regulation and systems biology*, 5, 75-88, 2011
- Kameda, T., *et al., Proc. Natl. Acad. Sci.,* 103 (47), 17765-17770, 2006

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Technology for metabolic design & optimization:
Toward the design of high-producing microorganisms for target compounds

RIKEN, Koto University, AIST

Common Platform Technology

Technical Description

A rational and theoretical metabolism must be designed in order for the microorganism to produce the targeted compound with high productivity. We have developed the following metabolic design tools.

1) Tool for predictions of artificial metabolic reactions: M-Path, BioProV

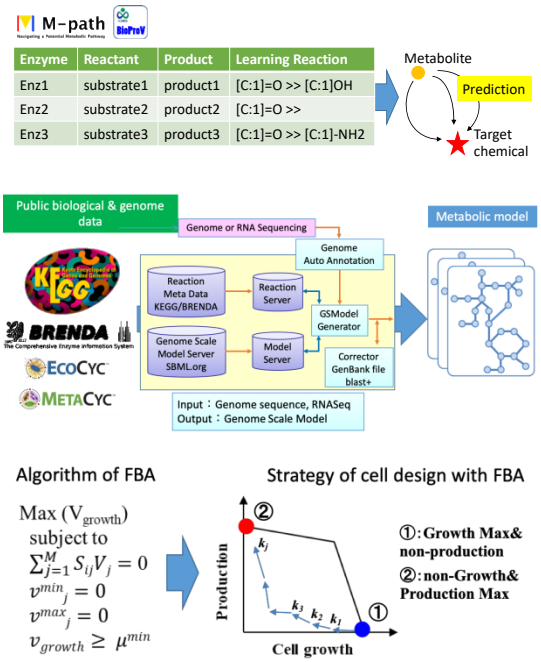
If the compound of interest cannot be synthesized by a biological reaction (pathway unknown), we can use them for the design of biosynthetic reactions. Based on the enzyme reaction database, let the computer learn each enzyme reaction pattern, and use it to predict and design artificial metabolic reactions.

2) Genome scale model generator

Based on the genome information of a host cell to produce a target compound, it is possible to construct intracellular metabolic reactions (genome scale model: GSM). We can construct a metabolic reaction model semi-automatically based on the acquisition of genome information with a next-generation sequencer and a metabolic reaction database such as KEGG.

3) Proposal tool for gene/reaction modification

GSM-based intracellular metabolic flux prediction technology (flux balance analysis: FBA) enables us to propose gene/enzyme reactions for high production of target compounds in microorganisms. A unique metabolic design algorithm using linear programming enables rapid metabolic design that achieves high production of the target compound.



Applications

- Realization of useful compounds from fossil materials using biosynthesis/searching for unknown metabolic reactions
- Calculation the theoretical yield of the target compound in consideration with energy and redox balance
- Proposal of gene/enzyme reaction modification sites for high productivity of a target compound

References

- Araki, M. et al., Bioinformatics, 31(6), 905-911 (2015)
- Shirai, T. et al., Microb. Cell Fact., 15(13), 1-6 (2016)

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Enzyme selection:

Toward the realization of metabolic design

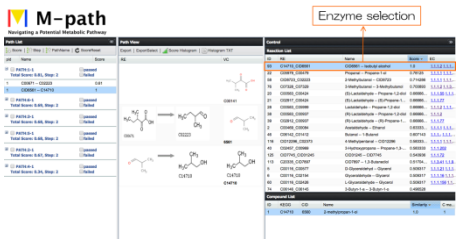
Kyoto University, NIBIOHN, RIKEN, AIST

Technical Description

In order to realize metabolic design, selection of enzyme genes in metabolic pathways is important. We are developing the following enzyme selection methods.

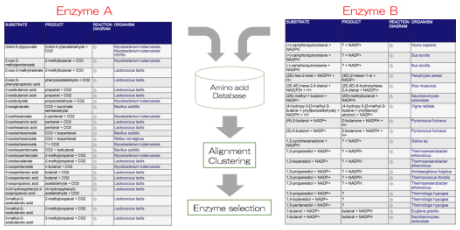
1) Metabolic design tool: M-Path

M-path is a system that presents enzyme candidate genes in each designed metabolic pathway. By presenting enzyme gene candidates in designed metabolic pathways based on an enzyme reaction database, we give guidelines for implementing the metabolic pathway.



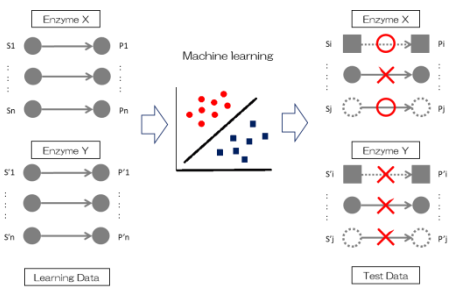
2) Bioinformatics tools

Enzyme sequences having similar EC numbers depend on the presence of the host/isozyme from which it is derived, and multiple enzyme gene sequences can be candidates. We are developing an enzyme gene selection method using bioinformatics and clustering.



3) Machine learning tools

This is a very useful method to find new enzyme reactions by performing machine learning based on known enzyme reaction data. We are developing a machine learning method that takes into account the combination of substrate/product and enzyme amino acid sequence.



Applications

- Searching for unknown enzyme reactions and genes for metabolic design
- Searching for enzyme genes to improve productivity of target compounds
- Refinement/advancement of metabolic models for theoretical yield calculation

References

- Araki, M. et al., Bioinformatics, 31(6), 905-911 (2015)
- Watanabe, N. et al., Journal of Chemical Information and Modeling, 60(3), 1833-1843 (2020)

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Gene regulatory network Modeling:

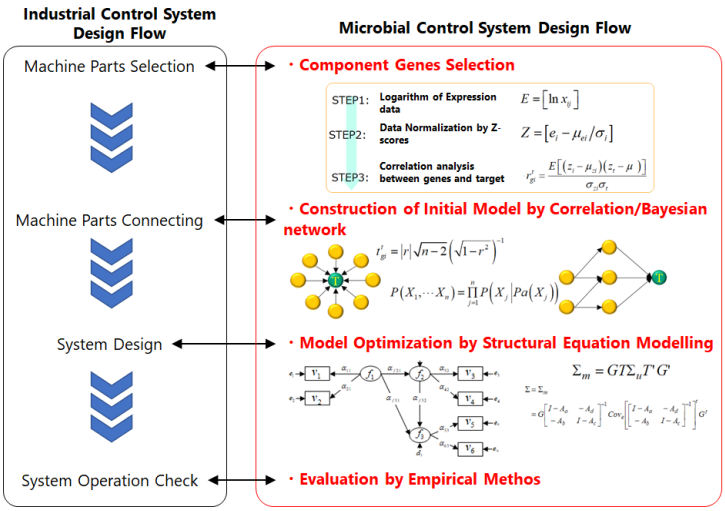
Inference of regulatory factors for bioproduction

AIST, RIKEN, Kyoto University

Technical Description

In the bioproduction field, improvement of productivity is one of the important themes. Usually, we utilize some empirical methods to improve the microbial productivity, but these methods need long terms and incur high costs. In this project, we developed a computational approach to infer the regulatory factors for microbial production.

To improve the microbial ability for bioproduction, clarification of mechanisms when the host microorganism produces the target compounds is important. We applied statistical network modelling methods, based on Structural Equation Modelling, to reveal the mechanism of bioproduction systems in this project. By using this method, we can obtain causal relationships between genes and productivity as a visualized graph. This graph indicates regulatory factors for target productivity (production amount of target substance, number of cells, etc.), and bottleneck points within the bioproduction systems.



Our developed methods are useful for improvement the productivity in microbial bioproduction. We utilize the entirety of gene expression data for modelling, thus we can find unknown regulatory factors. This method will be one of the new and systematic approaches for microbial breeding in the bioproduction field.

Applications

- Suggestion of genetic modification for improvement of microbial productivity
- Suggestion of genetic modification to control the balance of some compounds
- Suggestion of genetic modification to maintain growth level in host microorganisms

References

- Aburatani, S. et al., *Journal of Physics: Conf. Ser.*, 1391(1), 012043 (2019)
- Aburatani, S. and Toh, H., Encyclopedia of Information Science and Technology, 3rd Edition, Chapter 44, 458-467, IGI Global, (2015)

Contact Information

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Gene sequence design:

Technology to increase protein expression levels and for enhancing enzyme activity

AIST, Tohoku Univ., Kagoshima Univ., Sinshu Univ. and Okayama Univ.

Technical Description

1） Increasing protein expression levels

Codon optimization by synonymous substitution is widely used for recombinant protein expression. Recent studies have investigated sequence features for codon optimization based on large-scale expression analyses. However, these studies have been limited to common host organisms such as Escherichia coli. Here, we developed a codon optimization method for Rhodococcus erythropolis, a gram-positive. We optimized the coding sequences of 12 genes regarding these sequence features, and confirmed that 9 of them (75%) achieve increased expression levels compared with wild-type sequences (Fig.1) [1]. We also confirmed the effectiveness of our codon optimization method in other bacteria.

2） Enhancement of enzyme activity and protein heat resistance

We developed a method for enhancing enzyme activity using molecular dynamics (MD) simulation. Enzyme acts as catalyst of chemical reaction; it binds substracts and produces reaction products. We investigated the structural ensemble of enzyme-substracts binding complex forms using MD simulation (ALSD method) [2]. Based on structural information, effective mutants were predicted. These mutants had higher activity, confirmed by experiment (Fig.2). We also succeeded in designing protein mutants with higher heat resistance using MD simulation.

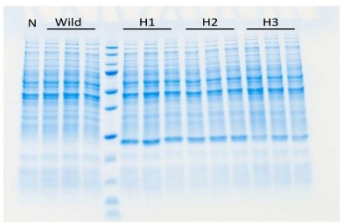

Fig. 1
Protein expression level increase (H1-3)

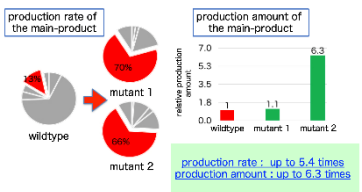

Fig.2
Enzyme activity enhancement using MD simulation

Applications

- Improvement of microbial productivity in the bioproduction field

References

- Saito Y. et al., Sci Rep. 2019; 9: 8338
- Ikebe J. et al., J. Comput. Chem. 2014; 35:39-50

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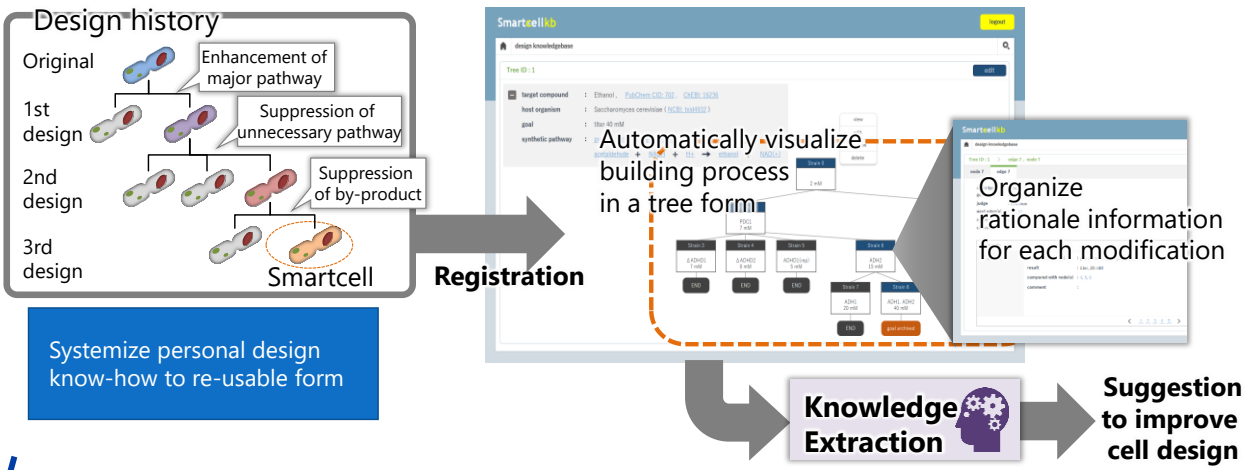
Smart cell design knowledge base:
Organizing information necessary for microbial design

Kyoto University, Hitachi, Ltd., Kyushu University

Technical Description

We are developing information systems that organize and systematize the decision-making, reference information, and experimental results in each step of the development process of smart cells.
The developed system aids in the organization of personal knowledge about microbe design into a reusable form. The system organizes and accumulates the design history of microbial strains and the genetic modification content of each strain associated with it in terms of purpose, means, and rationale information. Furthermore, by visualization of such accumulated information in a tree form along the design history, the system provides support for creating new hypotheses by taking a bird's-eye view of the whole history.
In addition, the system calls up the knowledge extraction techniques which works together from the accumulated and systematized design history, and presents useful information which leads to design improvement.



Applications

- Organizing, systematizing, and reusing various kinds of information associated with the development history of microbial strains

References

- Araki, Ito, Hanai, Bioscience and Industry (B&I), Vol.78(2), 168-169 (2020) (in Japanese)

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https://www.kyoto-u.ac.jp
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Microbial design knowledge extraction:

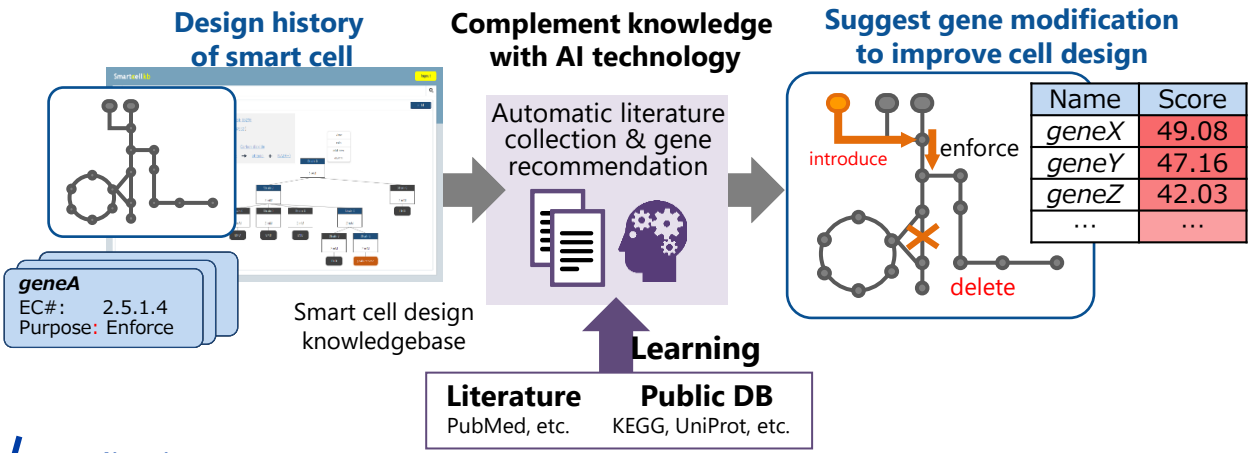
Suggesting gene modifications using AI technology

Kyoto University, Hitachi, Ltd.

Common Platform Technology

Technical Description

We are developing AI technology that extracts useful knowledge for microbial design from literature and public database and proposes potential genetic modification for productivity improvement.
The developed AI technology will contribute to the reduction of development periods of high-productivity microorganisms by complementing the design knowledge such as metabolic system modifications that has been overlooked in the design history of existing microbial strains. The features of the developed AI technology are as follows.
Automatic collection of smart cell literature: Identifies characteristics of literature related to metabolic design and gene modification and collects widely useful literature information for smart cell design.
Recommendation of promising gene modifications: Extract sand suggests the promising genetic modification related to the user's metabolic design from the collected literature information



Applications

- Explorer metabolic design and gene modification aimed at improving the productivity of target compounds

References

- Japanese patent application 2019-210138
- Japanese patent application 2020-45980

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Estimation of enzymatic activity:

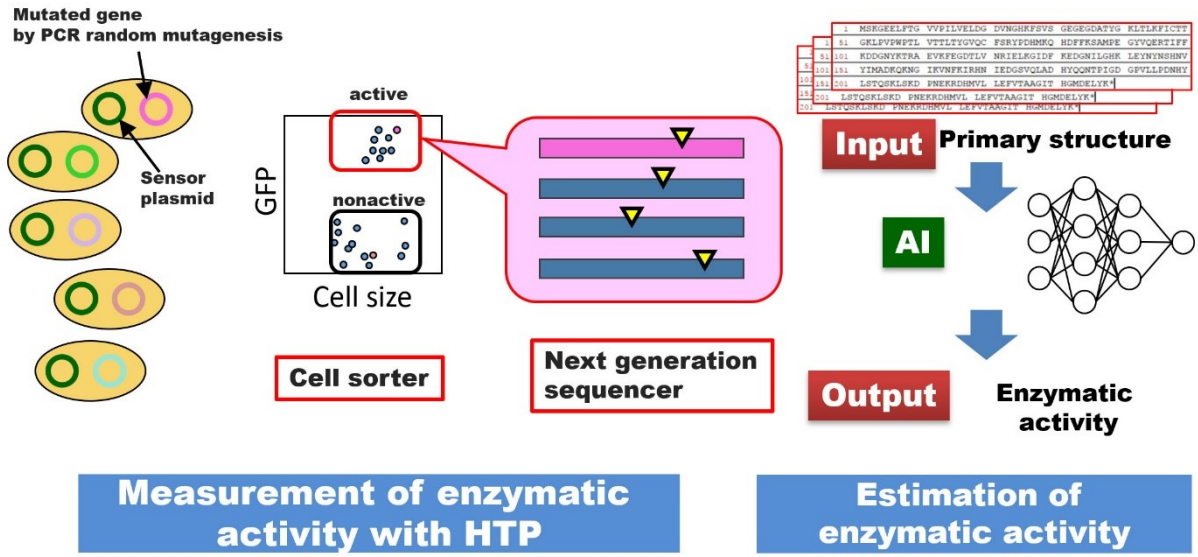
For design of enzymatic genes using AI

Kyushu University, Hitachi, Ltd. , Kyoto University

Common Platform Technology

Technical Description

We are developing a biosensor that can indicate enzymatic activity by the amount of fluorescence, and we are developing a system that can screen a large number of mutants by combining it with a cell sorter. By sequencing the mutants obtained from this system with a next-generation sequencer, it has become possible to obtain, for example, a large number of sequences of active mutants at one time. From this sequence information, we are developing an AI program to determine what kind of sequence is active or not.



Applications

- Estimation of enzymatic activity for bioproduction

References

- Umetsu, Hamada, Hanai, Proceedings of Kyushu Branch Conference, the Society for Biotechnology, Japan, p. 48 (2019)

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Bio-resource database:

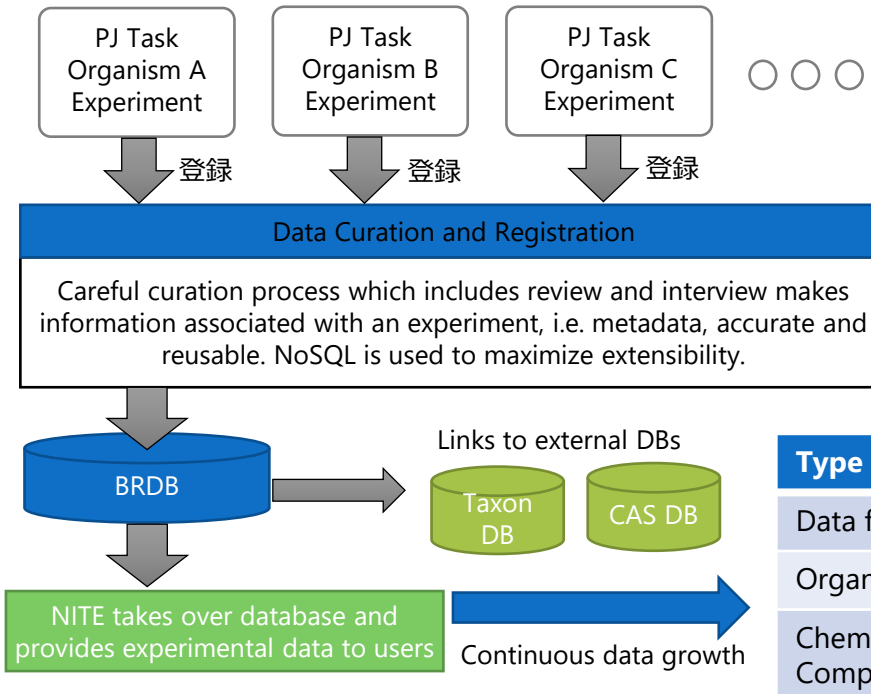
Large-scale experiment data collection

AIST, NITE

Technical Description

A large amount of experimental data from this project is curated and registered!

Turn experimental data into a reusable knowledge resource



Multi-Omics Data
Microarray
DNA-seq
RNA-seq
GC-MS
LC-MS
Data analysis

(As of Aug. 2020)

Type	No. of Items
Data files	2657
Organism	189
Chemical Compound	297

Applications

- An aid in your research for in-house breeding of micro-organisms to produce a target material
- A data resource to make your metabolic pathway model richer and more accurate
- A data resource to select your target material/target genes
- A dictionary of practical examples which helps you to start your own large-scale bio-data analyses

References

- NEDO Smart Cell Project: https://www.jba.or.jp/nedo_smartcell/project/

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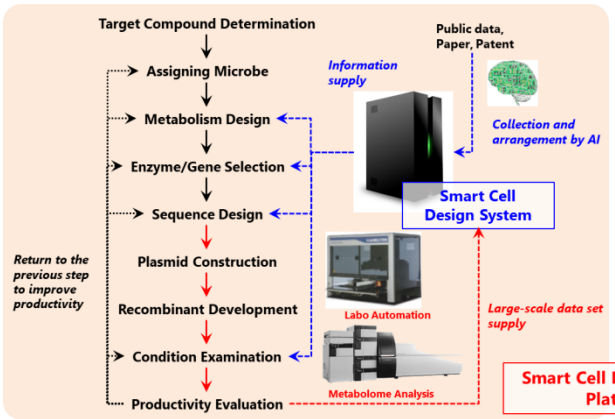
Wet overview:

Development of high-throughput synthesis/analysis/ evaluation technology

Technical Description

In the DBTL workflow (Figure), designing a smart cell requires a wide variety of data sets obtained from various microbial strains. Therefore, we are working on a high-throughput synthesis/analysis/evaluation technology that can build a diverse microbial library in a short time and acquire productivity data and omics data of target substances with high accuracy and high throughput. Among them, technologies for synthesizing long-chain DNA with the world's highest accuracy that can control the expression of many genes with one gene recombination operation, and metabolomics technology that can comprehensively measure the amount of metabolites with high reproducibility have a high advantage.



DBTL workflow of smart cell creation platform

Smart cell can be created in a short period of time by the high-throughput synthesis/analysis/evaluation technology (red arrow).

So far, we have succeeded in 1) establishment of technology for synthesizing long-chain DNA of more than 30 kb accurately (mutation rate 0.1% or less) at low cost (5 yen/base) within 1/4 or less of the conventional period (about 2 weeks), 2) construction of semi-automatic high-throughput transformation technology in 96-well plate format, 3) development of a platform to search for compound efflux transporters, 4) development of technology to evaluate the productivity of target substances at high throughput by image analysis, 5) development of a microbial proteome analysis technology that can quantify proteins, and 6) constructing a metabolome analysis system with high throughput, accuracy, and comprehensiveness by developing a pretreatment robot.
In addition to promoting the incorporation of elemental technologies into the smart cell creation platform, we are improving the accuracy of our original high-throughput evaluation technology and strengthening the cooperation with information analysis technology to systematically acquire and manage data.

References

- Nature Focal Point
- Japan Bioindustry Association website

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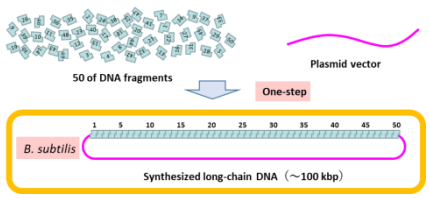
Total system for long DNA synthesis using the OGAB method:
From chemical synthesis to large preparation

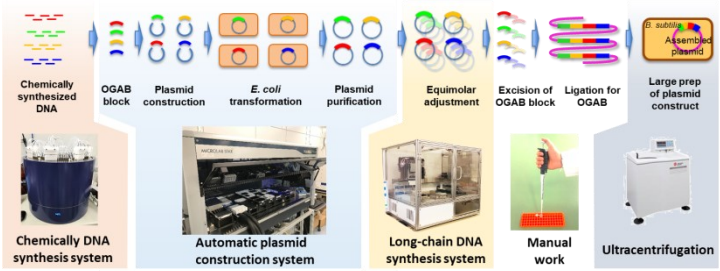
Kobe University

Technical Description

For smart cell construction, de novo synthesis technology of >10 kb of long-chain DNA is prerequisite. At Kobe University, we have developed the OGAB method, a gene assembly method that enables the connection of over 50 DNA fragments using Bacillus subtilis at one time. However, it takes 2 to 3 months to construct long-chain DNA exceeding 10 kb, which has been a bottleneck in the construction of smart cells. Conventionally, we outsourced a DNA fragment of about 1 kb, which is a material for the OGAB method, to a synthesis service, but it takes a long time of 1 to 2 months until all the DNA fragments of the material are available. In order to shorten this time, we constructed a total system for long-chain DNA synthesis, in which all steps from chemical synthesis of DNA are carried out by ourselves. By devising the design of the single-stranded DNA sequence and the process of converting it into double-stranded DNA, the process using a robot can be automated to rapidly prepare DNA fragments that are the material for gene assembly. Therefore, it became possible to prepare long-chain DNA of about 30 kb in 2 weeks.



OGAB method



Total system for long-chain DNA synthesis

Applications

- Rapid construction of smart cells
- De novo designing of novel metabolic pathways

References

- Tsuge, et al., Nucleic Acid Research, 31, e133 (2003)
- Tsuge, et al., Scientific Reports, 5, 10655 (2015)

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Development for preparation of long-chain DNA materials:

Development of new DNA synthesizer suitable for preparation of long-chain DNA materials

Nihon Techno Service Co., Ltd.

Technical Description

A high-throughput synthesis method for constructing material-producing strains is essential in development of smart cells, and it is essential to develop a long-chain DNA automatic synthesizer for producing long-chain DNA for more than 30 kb.
Long-chain DNA automatic synthesizers synthesize long-chain DNA by connecting DNA fragments, but when the DNA fragments are short, the amount of synthesis increases, which creates a risk of synthesis errors. As long as all DNA fragments necessary for the synthesis of long-chain DNA are not prepared, even if one of DNA fragment is short, an integration of long-chain DNA fragment is impossible. That is, in terms of reliable synthesis of DNA fragments as materials, shortening of procurement time is a key point. In addition, the cost of long-chain DNA preparation is related to the cost of the initial material (DNA fragment) directly.

Therefore, we have developed a 96-column synthesizer that can synthesize DNA fragments over 200 bases as an initial material in the automation of long-chain DNA synthesis with low cost, high efficiency, and short time. It has a multi-channel liquid delivery mechanism that enables minute control of reagents and high-speed mixing of each reagent. It is also possible to keep high reaction efficiency with a small amount of reagent. As a result, we achieved our plan that synthesized 96 kinds of synthetic DNA of 200 bases at a material cost of 3 yen/base within 20 hours.

In addition, the developed liquid transfer system is equipped to our other synthesizer. By improving the program, it can also use various reagents. It will also be applicable to fields such as nucleic acid chemistry and nucleic acid medicine.

M-96-LD
DNA synthesizer for long-chain DNA materials

M-2-TRS
DNA/RNA synthesizer

Applications

- Those who want to prepare chemically synthesized DNA of about 200 mer, which is a material for long-chain DNA synthesis, in a short time and at low cost
- Those who want to synthesize chemically synthesized nucleic acids including artificial nucleic acids
- Those who need from several mg of chemically synthesized nucleic acid

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A technology to isolate target DNA clones:

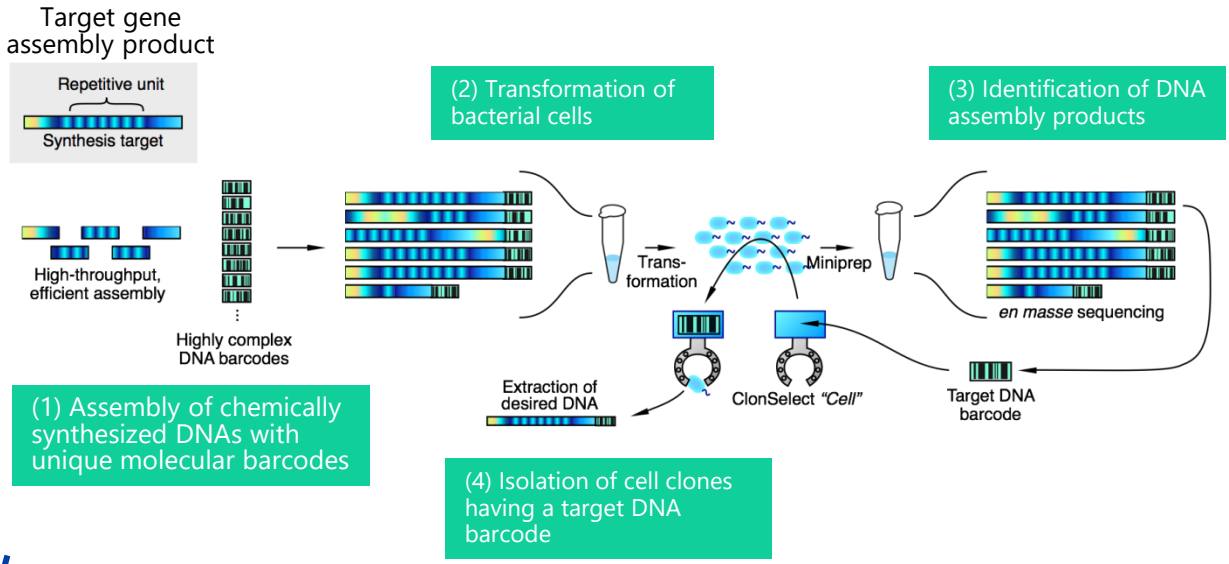
Developing a high-throughput gene synthesis platform

The University of Tokyo

Common Platform Technology

Technical Description

Any DNA assembly method is imperfect in its efficiency and requires bacterial transformation of a assembly reaction sample followed by clonal isolation and DNA sequencing of many samples to obtain a target product. This restricts us to obtain DNA products which assembly efficiencies are limited. We developed a new clone isolation technology where (1) DNA assembly products are tagged with unique molecular DNA barcodes, (2) transformed to bacterial cells, and (3) identified by massively parallel DNA sequencing. Cells harboring a target product can be then isolated from a heterogeneous cell pool by DNA barcode-dependent genome editing. This technology accelerates the conventional DNA assembly process by a factor of 1,000.



Applications

- DNA assembly
- Gene synthesis
- Automation of DNA assembly and gene synthesis pipelines

References

- Nishida K, Arazoe T, Yachie N, Banno S, Kakimoto M, Tabata M, Mochizuki M, Miyabe A, Araki M, Hara KY, Shimatani Z & Kondo A. Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems. (2016) Science 353, aaf8729

Contact Information

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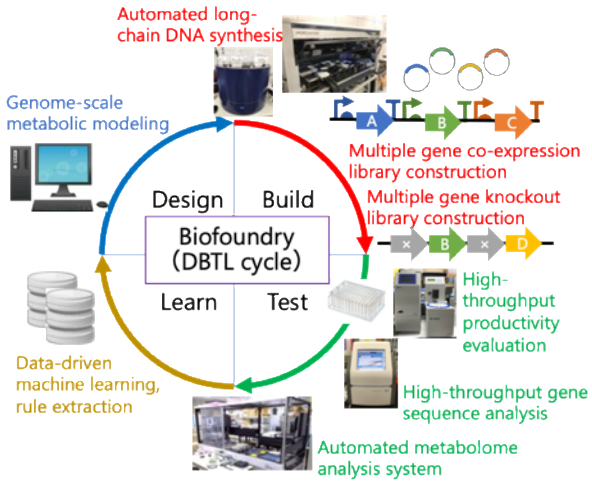
Ultra-rapid development of chassis strains:

The smart cell workflow for accelerating creation of smart cells

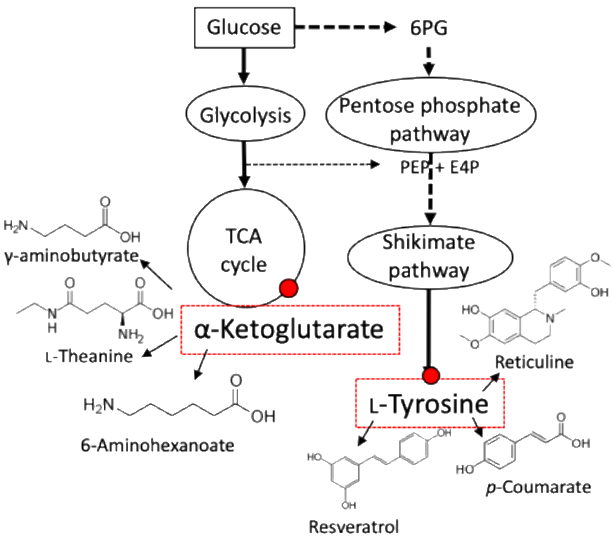
Kobe University

Technical Description

Valuable chemicals can now be biosynthesized through key metabolic precursors referred to as hub compounds. To speed up the accumulation of hub compounds, chassis strains are engineered as a type of smart cell with increased metabolic flux to the target metabolite. The smart cell workflow developed through the smart cell project enabled faster construction of L-Tyrosine or α-ketoglutarate *Escherichia coli* chassis strains, within a development period of 2.5 months. Our approach has been successfully applied to rapidly developed smart cells for production of downstream targets derived from the hub compounds (α-ketoglutarate: γ-aminobutyrate, L-theanine, and 6-aminohexanoate; L-Tyrosine: reticuline, *p*-coumarate, and resveratrol).



The Smart-cell workflow



Applications

- Those who desire ultra high-speed chassis engineering
- Those who are thinking of bio-production of downstream targets of α-ketoglutarate and L-Tyrosine

References

- Smart Cell Industry —Prospect of Bio-Based Material Production Using Microbial Cells— (2018)
- Vavricka CJ *et al.* Nat Commun. 10(1):2015 (2019)

Contact Information

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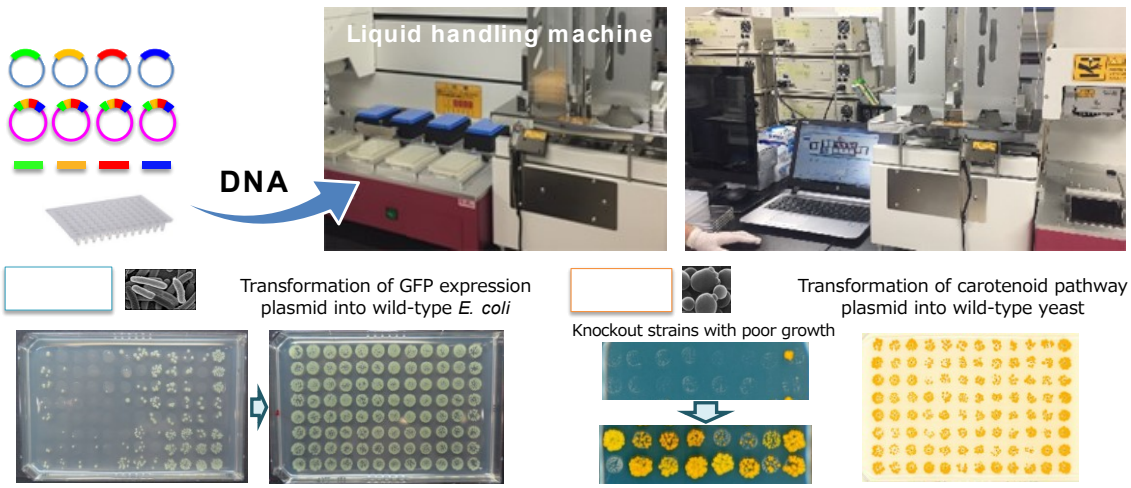
High-throughput construction and evaluation of engineered microbes:

An automated transformation system using a liquid handling machine, and high-throughput analyses

Kobe University

Technical Description

We developed an automated transformation system for introducing plasmid DNA into *Escherichia coli* or yeast in a 96-well format by originally customizing the program of a liquid handling machine and conditioning the transformation method. This made it possible to generate several thousand or more different transformants at once. In addition, we also constructed high-throughput analytical methods and simple assay systems for target compounds, enabling the rapid evaluations to estimate the productivities of a vast number of transformants. These fundamental technologies permit rapid construction of microbes with various genotypes and evaluation of their productivities.



Development of an automated transformation system for *E. coli* and yeast using a liquid handling machine

Applications

- Companies that aim to commercialize bioproduction using *E. coli* and yeast
- Public institutions that aim to conduct basic research on *E. coli* and yeast

Our technology makes it possible to comprehensively search for new useful gene candidates that contribute to productivity improvement in strain development.

References

- CMC Publishing Co., Ltd. "Smart Cell Industries: Prospects of bioproduction using microbial cells" Part 1, Chapter 1, Section 1: Bioproduction using microorganisms and high-throughput technology for construction of engineered microbes

Contact Information

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Metabolite sensors:

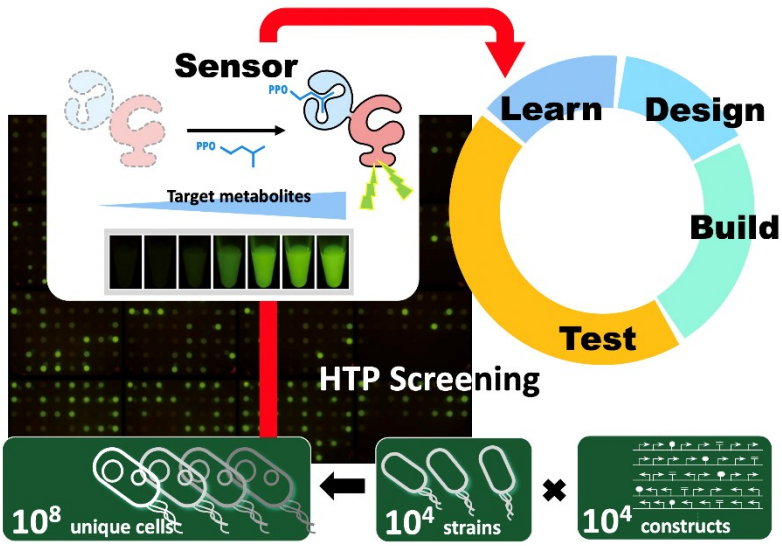
Any metabolites, with any sensitivity

Chiba University

Technical Description

This technology enables high-throughput screening of pathway/cellular variants with high level of targeted metabolites based on biosensors.

By visualizing the stability change of the receptor upon capturing metabolites, we can provide on-demand sensors for virtually any metabolites of your interest. Because we can quickly tune the sensor sensitivity, one can search high-performing variants. Also enabled is the ability to search for genetic elements/manipulations that can alter the cellular level of the metabolites of interest, thereby gathering a wealth of information that aids the molecular breeding of the given pathways/cells.



Applications

- Any individuals/organizations who want to establish new pathways toward valued chemicals
- Any individuals/organizations who want to improve existing pathways in yield
- Any individuals/organizations who want to evaluate the impact of genetic manipulations

References

- M. Tominaga, *et al.*, P*LOS ONE*, **10**, e0120243 (2015)
- Y. Kimura, *et al., ACS Synth. Biol.*, **9**, 567-75 (2020)
- Japanese patent 5959127, 5904494（US9,315,816）, 5757608, Japanese patent application 2018-057314

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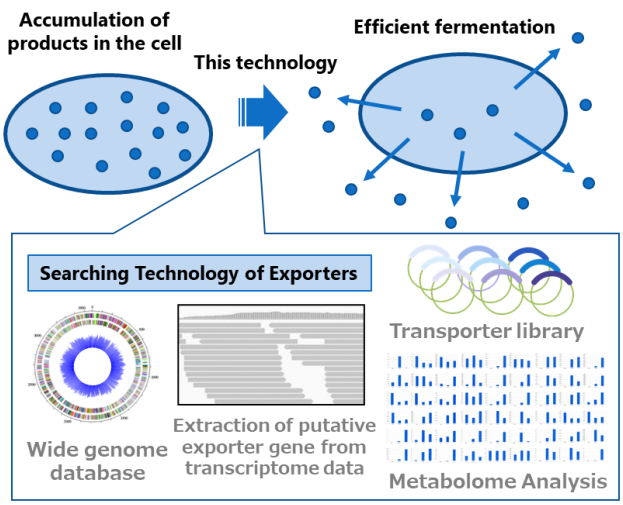
Platform for screening of industrially beneficial transporters

Tohoku University, AIST

Technical Description

Productivity of water-soluble products by fermentation is restricted by their polarity, which prevents them from crossing the cell membranes. Accumulation of products in the cell creates negative feedback regulation that inhibits biosynthetic reactions inside the cell. Membrane transporters are required for mediating products efflux. However, because membrane transporters are substantially more difficult to handle than water-soluble proteins, too little information is available to identify transporters for target molecules from genome databases. In fact, most metabolic pathway maps that represent current knowledge of reaction and relation networks do not include the functions of membrane transporters. We developed technology for searching transporters appropriate for product efflux to achieve efficient fermentation.

We developed technology to identify transporters by using stable expression methods for membrane proteins and mass spectrometry. First, we constructed an algorithm for scoring the putative transporter genes from gene sequences. The predicted transporter genes were cloned and the screening of the genes encoding exporters for target chemicals was conducted. We have identified and cloned novel amino acid transporters and a shikimate transporter using this method (Patents lists 1). Furthermore, we are developing technologies required for stable expression of exporters in cell membranes.



Applications

- People who have trouble with accumulation of target chemicals in cells
- People who have trouble with low yield of target chemicals after metabolic engineering

References

- Japanese patent application 2018-087700

Contact Information

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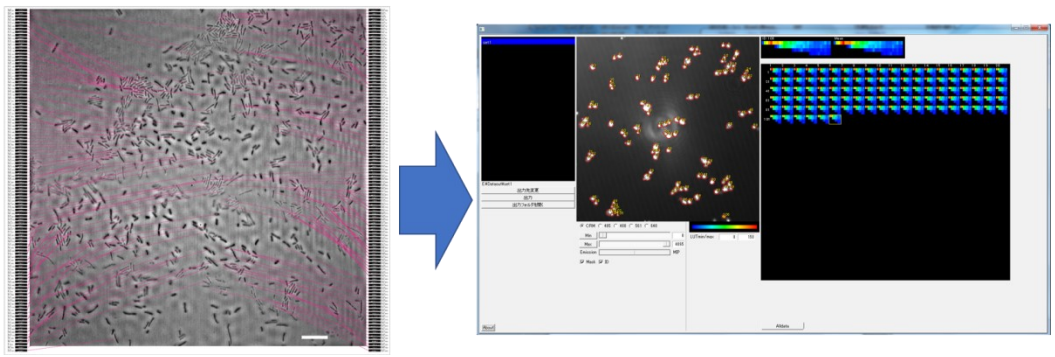
Innate fluorescence signature microscopy:

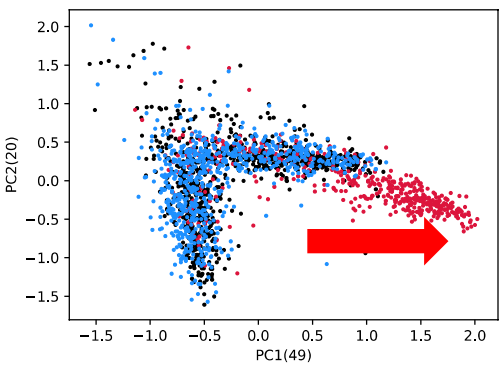
CRIF: confocal reflection microscopy-assisted single-cell innate fluorescence analysis

University of Tsukuba

Common Platform Technology

Technical Description





CRIF (confocal reflection microscopy-assisted single-cell innate fluorescence analysis) is a technology used to non-invasively (i.e. optically) extract and catalogue autofluorescence signature of a single cell in a microbial cell population. The library of the single-cell autofluorescence signature allows prediction of a cell's physiological status at a resolution of single-cell.

Applications

- High-throughput screening
- Those who need an objective index for quality control of cultured cells

References

- Yawata Y. (co-corresponding author) , T. Kiyokawa , Y. Kawamura , T. Hirayama , K. Takabe , N. Nomura. 2019. Intra and inter species variability of single-cell innate fluorescence signature of microbial cell, Applied and Environmental Microbiology 85, e00608-19
- Japanese patent 6422616

Contact Information

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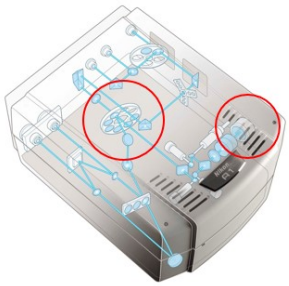
Autofluorescence spectrum observation using confocal laser microscope:

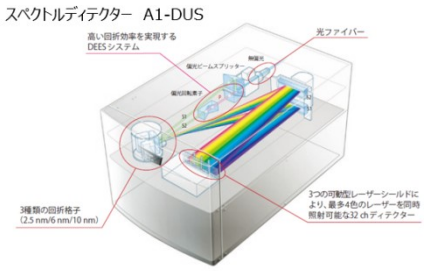
Accurate spectrum detection with one wide–field shot

Nikon Instech Co., Ltd.

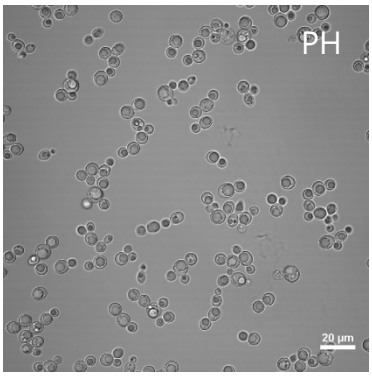
Technical Description

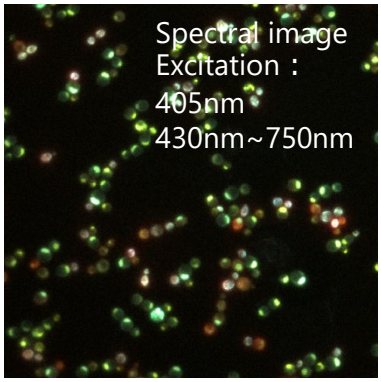


左：大口径スキャンレ
右：従来スキャンレンズ



We can efficiently detect weak autofluorescence using a confocal laser microscope (32-ch A1RHD25), and separate stray light and reflected light from fluorescence and lasers.





Though the bacterial population look uniform by PH, we can now see their variety.

Applications

We can detect the weak autofluorescence spectrum of microbial cells with one wide–field shot.

References

Strain offer: Takaku Laboratory (Niigata University of Pharmacy and Applied Life Science)
Photo: Yahata Laboratory (University of Tsukuba)
Collaborator: Prof. Nomura (University of Tsukuba)

Contact Information

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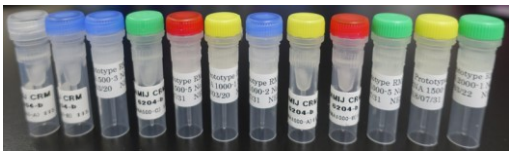
Improved transcriptomics

National Institute of Advanced Industrial Science and Technology (AIST)

Technical Description

In order to establish reliable transcriptome analysis technology applicable to various industrial microorganisms, we will develop nucleic acid reference materials for spike-ins and validate their use. We will also establish a simple method of valuing the nucleic acid reference materials for this purpose.

In industrial microorganisms, there are cases where the production of target useful substances becomes high, especially in the late stage of culture, but it is difficult to extract RNA from such samples in good condition. We are developing a technique to selectively analyze only undegraded RNA for transcriptome analysis, which has been difficult to perform until now.

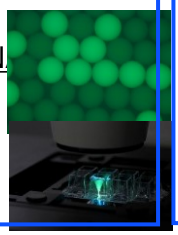


Development of standard RNA

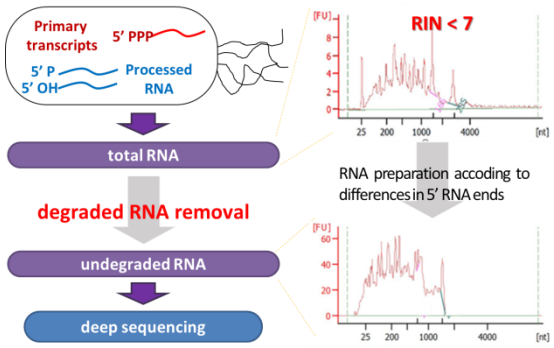
- Long version of spike-in RNA
- GC variation of spike-in RNA

Quantification of standard RN

- Digital droplet PCR
- Single cell quantification (FCS)



Terminator-5'-phosphate-dependent exonuclease (TEX) selectively digests processed transcripts, which results in an enrichment for primary transcripts.



Applications

- RNA-Seq analysis using next-generation sequencers
- Expected to be particularly effective in cases where it is difficult to recover good RNA in the late stage of culture when material production increases
- Compatible with both prokaryotic and eukaryotic organisms

References

- Absolute quantification of RNA molecule using fluorescence correlation spectroscopy with certified reference materials. Anal. Chem. (2018)

Contact Information

Bioproduction Research Institute, AIST

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Development of high-precision metabolome analysis technology 1:
Automated sample preparation system for metabolome analysis

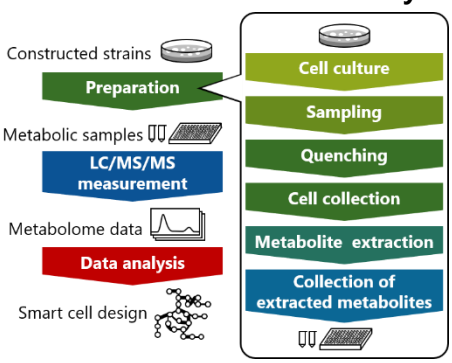
Kobe University, Shimadzu Corporation

Technical Description

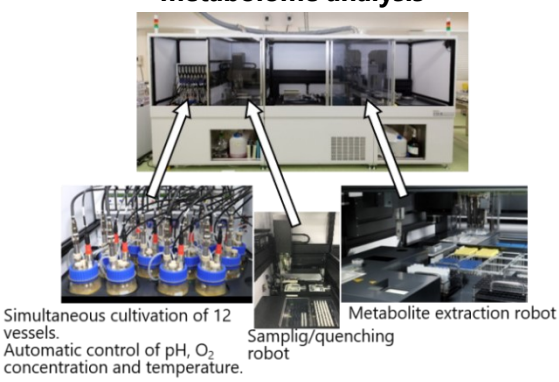
Metabolome analysis is a technique for simultaneously clarifying the numerous amounts of metabolites existing in cells. Since metabolome data reflects the growth environment and genetic background of cells, metabolome analysis enables grasping of culture conditions and cell states suitable for target metabolite production, and identification of bottleneck reactions in metabolic pathways. Also, with comprehensive metabolome analysis it becomes possible to clarify the causal relationship between the production capacity of cells and genetic recombination.

In this research and development, in order to improve the complex problems of sample preparation, which is slow and difficult to reproduce by hand (Fig. 1), a robotic "automatic preparation system" was developed to automatically perform the preparation process (Fig. 2). This system is equipped with 12 automatic culture vessels and a robot for culture sampling and quenching of metabolism, enabling continuous collection of multiple samples, a process that is difficult to perform by hand. The metabolite extraction robot shortens the processing time that usually takes an expert 180 minutes down to 75 minutes. Since it can operate overnight, this robot realizes high-throughput capability equivalent to approximately 20 human operators. Robotization improves not only the throughput but also the precision. With this system, anyone can realize a highly reproducible preparation process that is equal to or higher than that of an expert. In addition, the robot prevents sample and data mixups that often occur by hand by implementing a barcode system for sample/data management.

1. Workflow of metabolome analysis



2. Automated sample preparation system for metabolome analysis



Applications

- High precision measurement of intracellular and extracellular metabolites
- Tracking and quantifying time course profiles of metabolite in culture/fermentation by processing multiple samples

References

- Vavricka, C.J., et al., Dynamic metabolomics for engineering biology: Accelerating learning cycles for bioproduction, Trends in Biotechnology, 38(1):68-82. (2019)
- Japanese patent application 2018-134171, 2018-134174, 2018-134169, 2018-134177, 2018-134179
- Shimadzu Corporation press release, 25 May 2018

Contact Information

Engineering Biology Research Center, Kobe University
E-mail: hasunuma@port.kobe-u.ac.jp **URL:** http://www.egbrc.kobe-u.ac.jp/index.html

Development of high-precision metabolome analysis technology 2:
High-precision metabolome analysis, high-throughput evaluation system

Kobe University, Shimadzu Corporation

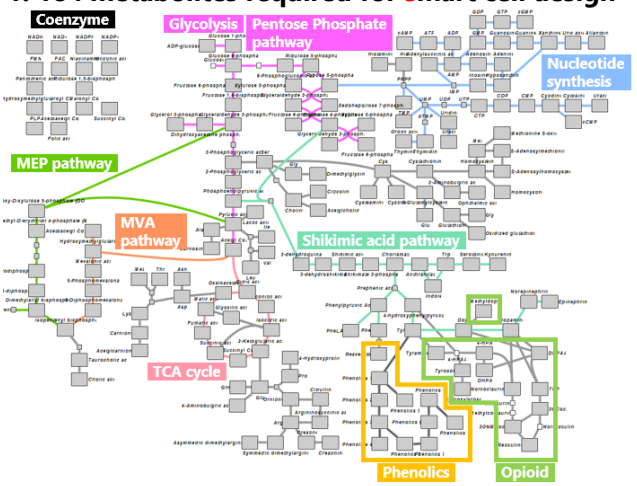
Technical Description

To better separate and detect metabolites, an LC/MS/MS system was developed without relying on an ion pair regent to improve the S/N ratio. This system enabled simultaneous separation/detection of 184 water-soluble metabolites required for smart cell design.

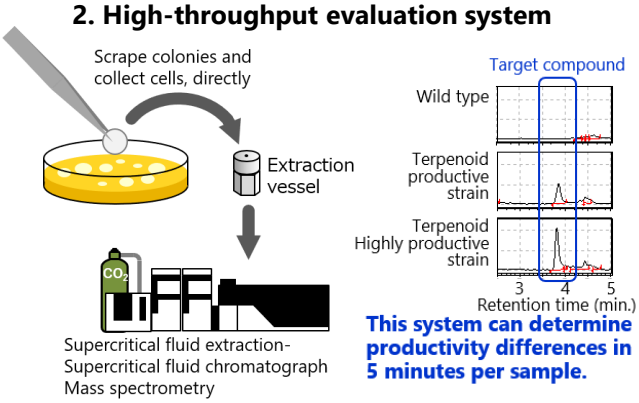
For metabolome analysis, a large amount of data must be processed to identify and quantify many metabolites in each sample. Therefore, a data analysis system was developed to support peak picking from chromatograms and extract the results into metabolic maps. These systems make it possible to obtain a large amount of highly reproducible data and contribute to the elucidation of the metabolic control mechanisms.

Complex preparation and workup of pre-cultures and main cultures is usually required for the analysis of intracellular and extracellular metabolites. Using conventional methods it takes a long time to screen target compounds in engineered smart cell systems. Therefore CO_2 supercritical fluid extraction was developed to extract metabolites from a small amount of cells with high efficiency. In this research and development, CO2 Supercritical fluid extraction was applied to directly analyze microbial colonies without any required workup. This system can detect target metabolite productivity differences in 5 minutes per sample, and therefore can perform screenings that normally take days to weeks in just a single day.

1. 184 metabolites required for smart cell design



2. High-throughput evaluation system



This system can determine productivity differences in 5 minutes per sample.

Applications

- High-precision measurement of intracellular and extracellular metabolites
- Rapid screening of engineered microbes to mass produce valuable compounds

References

- Hasunuma, T. et al., Temperature enhanced succinate production concurrent with increased central metabolism turnover in the cyanobacterium Synechocystis sp. PCC 6803, Metabolic Engineering, 48, 109-120
- Japanese patent application 2018-207920

Contact Information

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Quantitative targeted proteomics

Measurement of protein levels in your samples

Osaka University

Technical Description

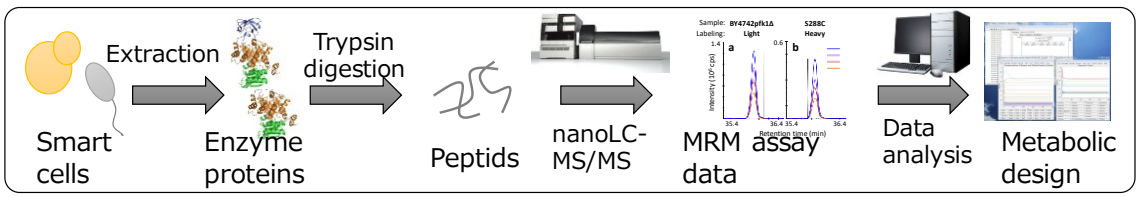
Quantitative targeted proteomics enables you to:

・Measure concentration of multiple proteins produced by microorganisms
・Check expression levels of enzymes in a metabolically-engineered microorganisms
・Identify bottlenecks in metabolic pathways

This is because:

・ Only amino acid sequences of target proteins are required for the liquid chromatography-mass spectrometry (LC-MS) base
・Preparation of antibodies is unnecessary
・Up to 20 proteins can be measured in one analysis
・LC-MS method database for industrial microorganisms is ready for use

- Trypsin digested-peptides of target proteins are measured by LC-MS
- Better sensitivity and selectivity than the electrophoresis-based methods



Applications

- For enzyme production: Measurement of multiple proteins produced by microorganisms
- For metabolic engineering: Confirmation of enzyme over-expressions in a metabolically-engineered microorganism
- For metabolic engineering: Identification of metabolic bottleneck by comprehensive measurement of central metabolism-related enzymes.

References

- F. Matsuda, A. Tomita, H. Shimizu, Prediction of hopeless peptides unlikely to be selected for targeted proteome analysis. Mass Spectrometry 6, A0056 (2017).
- F. Matsuda. Quantitative targeted proteomics. Satoru Kuhara Ed. Smart cell industry. Prospect of bio-based material production using microbial cells pp69

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Chapter 2

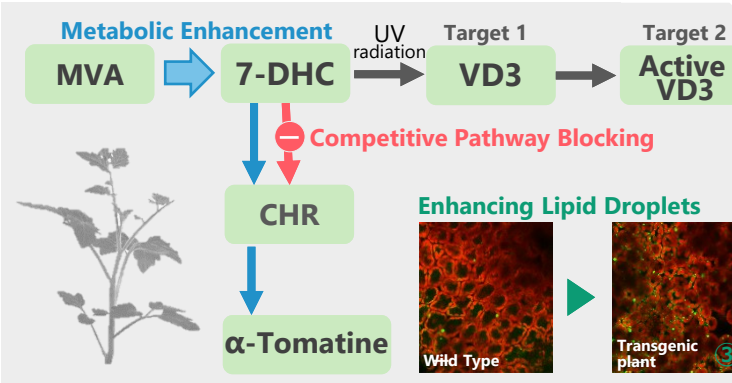
Verification of platform technology in material production

Development of a vitamin D3 production system using multi-step metabolic engineering and highly efficient plant tissue culture

Takenaka Corp., Kirin Holdings Co. Ltd., KNC Laboratories Co. Ltd., Osaka University, Osaka Prefecture University, Kobe University, Health Sciences University of Hokkaido

Case Description

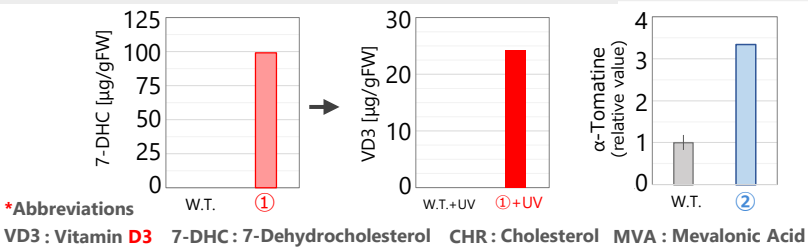
Vitamin D3 is used as an effective treatment for osteoporosis. It is reported to occur only at trace levels in certain plant species.
In this project, we try to increase the yield of vitamin D3 production using multi-step metabolic engineering. As a result, drastic improvements in vitamin D3 precursor content ratio, etc. were achieved. Moreover, we will establish a production system using bag-type bioreactors. Then we will build next-generation plant factories for the efficient supply of plant-origin substances.



①Drastic improvement in 7-DHC content ratio by competitive pathway blocking

②Enhancing whole metabolic flux by modification of upstream genes

③Establishment of new method for enhancing lipid droplets in plant cells



*Abbreviations
VD3 : Vitamin D3 7-DHC : 7-Dehydrocholesterol CHR : Cholesterol MVA : Mevalonic Acid

Commercialization Plan

- STEP 1: Development of multiple transgenic plants
- STEP 2: Establishment of a high-efficiency plant tissue cultivation system using bag-type bioreactors
- STEP 3: Implementation of next-generation plant factories

References

- Transgenic Plants and Use Thereof, International Publication WO-A1-2019/163601

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https://www.kirinholdings.co.jp/
URL: http://www.kncweb.co.jp/

Alkaloid production using metabolic-engineered medical plants:

Modification of alkaloid content in *Catharanthus roseus*

Ajinomoto Co. Inc., Kyoto University, Chiba University, Tamagawa University,
Tokushima University, Northern Advancement Center for Science & Technology

Verification of platform technology in material production

Case Description

1. Research and development purpose

①To develop a technology platform for the production of raw materials of plant-derived medicines in plant factories.

②To increase the monoterpene indole alkaloid contents in *Catharanthus roseus*.

③To optimize the cultivation technique of *Catharanthus roseus* in closed artificial-lighting plant factories.

2. Introduction of cases

①To enhance tryptophan metabolism, we introduced *aroG4* and *trpE8D* genes (*AED* genes) into *Catharanthus roseus* using a transforming system that we developed. As a result, it was confirmed that the catharanthine content in the leaves of transformants increased (Fig.3).

②When the tryptophan metabolism-enhancing genes (*AED* genes) and the alkaloid transcription factor *ORCA4* gene were co-expressed in *Catharanthus roseus* using a transient expression system that we developed, the tabersonine content increased significantly (Fig. 4).

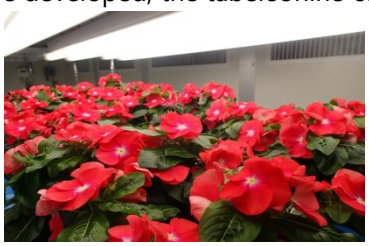

Fig. 1 The cultivation of *Catharanthus roseus* in closed artificial-lighting plant factories

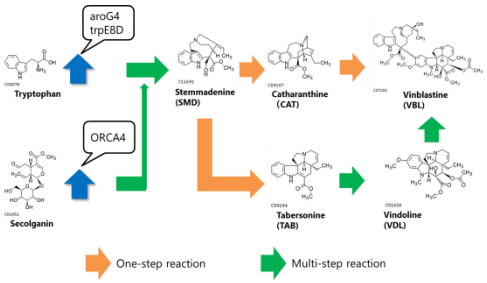

Fig. 2 Vinca alkaloid metabolic pathway in *Catharanthus roseus*. ①Enhanced expression of *aroG4* and *trpE8D* genes. ② Enhanced expression of *aroG4*, *trpE8D* genes and *ORCA4*.

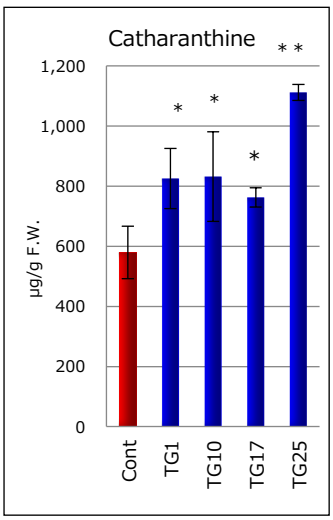

Fig. 3 Analysis of catharanthine content.
- Cont: non-transformed plant.
- TG 1 , 10, 17, 25: transformed plants introduced *aroG4-trpE8D* genes .

（n=3, *t*-test * P<0.05 **P<0.01 ）.

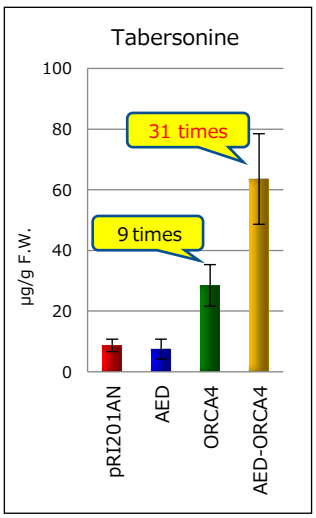

Fig. 4 Analysis of tabersonine content.
- pRI201-AN: vector control.
- AED: *aroG4-trpE8D* genes.
- ORCA4: *ORCA4* gene.
- AED-ORCA4: *AED* and *ORCA4* genes connected.

Commercialization Plan

- Using *Catharanthus roseus* as a versatile production platform for monoterpene indole alkaloids, we will produce and cultivate plants with increased content of various alkaloid biosynthesis intermediates.

References

- Method for producing alkaloids, WO2019-194309A
- Transformation of *Catharanthus* genus in *Apocynaceae* family. JP 2020-039277A

Contact Information

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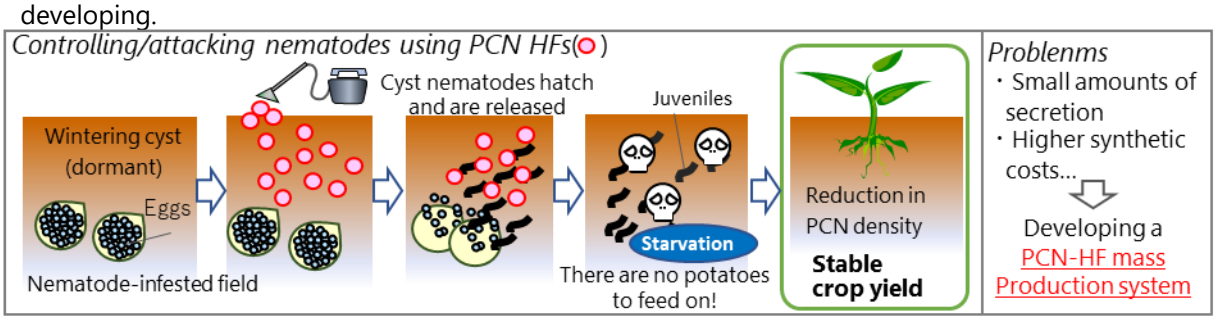
Production of substances which stimulate the hatching of potato cyst nematodes using transgenic solanaceous plants:
Development of new controlling agents against potato cyst nematodes

Hokusan Co., Ltd., National Institute of Advanced Industrial Science and Technology (AIST)

Case Description

Agriculture has suffered enormous damage worldwide due to increasing infestations of potato cyst nematodes (PCN). Currently, no effective countermeasures or control agents have been developed. Consequently, we have been developing a mass production system for PCN hatching factors (PCN-HF) with the intention of controlling the spread of the plant parasite.

PCN is a difficult-to-control pest that infests potato roots by responding to HFs secreted by host plants, allowing larvae to hatch from eggs. The panel below shows the control method we have been developing.



This PCN-HF mass production system has been developed by 1) exploring and evaluating metabolic genes related to PCN-HF biosynthesis through machine-learning models, 2) producing plants that yield high levels of PCN-HF through genetic manipulation, 3) optimizing PCN-HF high-production cultivation conditions using drug information indices, 4) examining PCN-HF recovery methods.

Table PCN-HF production comparisons (per cultivated area per year) between table cultivation methods.

Cultivation method	Ⓐ Number of collections/ cultivation	Ⓑ Nutrient solution volume			Ⓒ Relative hatchability	Ⓓ Number of cultivations/ year	PCN-HF score	Relative value (Conventional = 1)
		Nutrient sol. volume/ strain (L)	Strains/ m^2	Dilution ratio during hatching test				
Conventional method	4	0.40	39	1.25	1.30	3	304	1.0
Modified MT method	5	0.08	152	50.00	0.91	5	13,781	45.3

PCN-HF scores showing the amount of PCN-HF contained in the nutrient solution (Ⓐ×Ⓑ×Ⓒ×Ⓓ)
Relative hatchability: PCN hatchability upon treatment with $NaVO_3$ (a chemical synthetic with confirmed hatching activity) was set as 1

To date, the microtuber method has been modified to produce 45 times more PCN-HF than conventional soil cultivation as the table on the left shows.

Commercialization Plan

After the completion of a mass production system for PCN HFs, we plan to develop a new PCN control agent using the manufactured PCN HFs as the main component. We aim to produce and commercialize these materials for agriculture after the third year of completion of research and development.

References

- Analysis of the mechanisms regulating the expression of isoprenoid biosynthetic genes in hydroponically-grown *Nicotiana benthamiana* plants using virus-induced gene silencing. Sci Rep. 2018; 8(1):14804
- Method for obtaining sterilized cyst nematodes PCT/JP2019/005512 International filing date 15.02.2019

Contact Information

Hokusan Co., Ltd. Plant Biotechnology Center

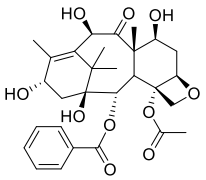
E-mail: noriko-tabayashi@hokusan-kk.jp **URL:** https://hokusan-kk.jp

Development of an efficient production system for the pharmaceutical intermediate 10-DAB using yew-tree cell culture technology

Hokkaido Mitsui Chemicals, Inc., Kyoto Univ.

Case Description

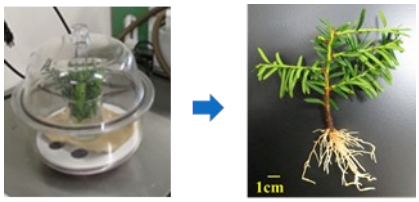
[Background]
Taxane-type anticancer drugs, paclitaxel, docetaxel and cabazitaxel are semi-synthesized using the intermediate 10-DAB, but its complex structure makes their supply using chemical synthesis impractical. Moreover, yew grows slowly and has a very low content of 10-DAB, which makes it difficult to provide a stable supply of 10-DAB.



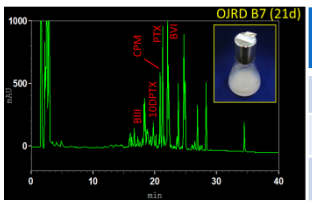
10-DAB

In order to develop an efficient production method for 10-DAB, the following four issues were examined.
1. Development of gene recombination technology in yew
2. Acquisition of a high-production method of taxane compounds
3. Control of transport and accumulation of taxane compounds
4. Development of a bag-type bioreactor

[Results]

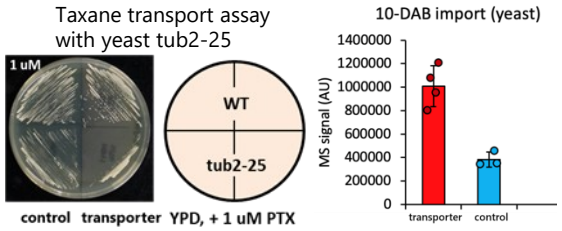


1. Efficient protocol for inducing hairy roots



	Taxane yield (mg/L)
Cells	449.7
Medium	28.9
Total	**478.6**

2. High-producing *Taxus* cell line for taxane compounds



3. Taxane transporter

4. Cell suspension cultures in single-use bag system

Commercialization Plan

- FY2022: Establishment of 2m^3 level single-use bag culture technology and achievement of 1g/L/28d 10-DAB productivity
- FY2024: Installation of bioreactor
- FY2025: 10-DAB production starts

References

- Japanese Patent Application No. 2019-53639
- Japanese Patent Application No. 2019-152191
- Kusano, H. et al, Evolutionary developments in plant specialized metabolism, exemplified by two transferase families, Front Plant Sci 10:794. DOI: 10.3389/fpls.2019.00794 (2019)

Contact Information

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Development of efficient production technology for functional ingredients of perilla:
Combination of gene manipulation and cultivation technology

Amino Up Co., Ltd., AIST, NOASTEC, Tokushima University

Case Description

Perilla frutescens

Importance of self-medication for an aging society and extension of healthy life expectancy
⇒ Increase in demand for products containing functional ingredients (functional foods)

Problems are...
- **Functional ingredients content**
- **Raw material yield**

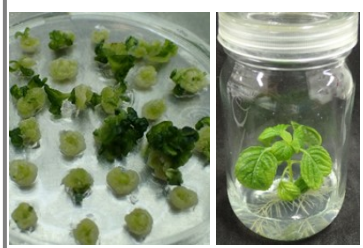
Unstable!!!

<Variation factors>
Weather, annual and regional differences

Development of technology for "stable" cultivation of perilla with a "high content" of functional ingredients

Achievement

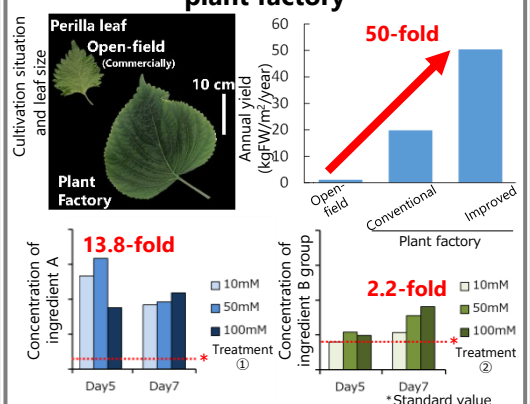
Genetic transformation / genome editing



Shoot formation | Regeneration of small plant

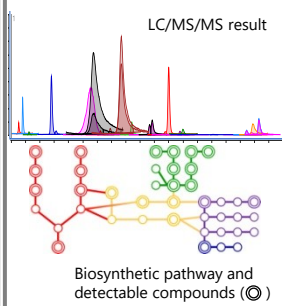
- Successfully generated genetically modified and genome-edited plants

Cultivation techniques in a closed plant factory



- Annual yield and the content of functional components increased

Simultaneous analysis of metabolites



- 23 related metabolites became detectable simultaneously

Commercialization Plan

- By combining genetic manipulation and cultivation techniques, produce perilla with a high content of functional ingredients in a plant factory
- Manufacture high-performance materials using above-mentioned perilla

References

- Method for cultivating plant and method for increasing rosmarinic acid content in plants
- Japanese Patent Application No. 2018-002334 (Amino Up Co., Ltd., AIST)

Contact Information

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Control of simultaneous production of useful proteins in filamentous fungi:

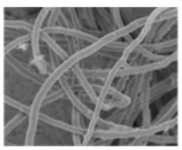
Expression control technology of multiple proteins

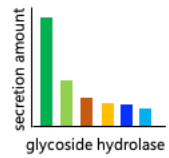
Nagaoka Univ. of Technol., Kao Corp., JBA, AIST, Kyushu Univ.

Verification of platform technology in material production

Case Description

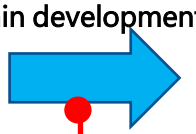
Trichoderma reesei



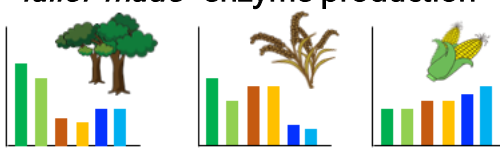


T. reesei produces a large amount of enzymes with a constant ratio.

Strain development

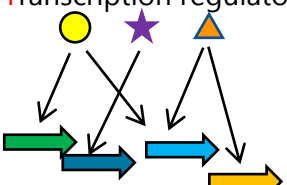


"*Tailor-made*" enzyme production



Enzyme cocktail with suitable ratio for each biomass

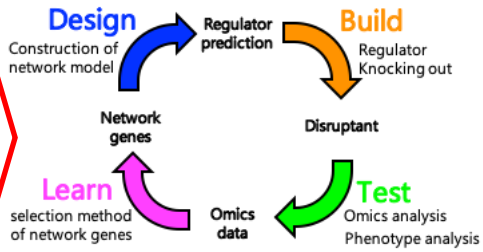
Transcription regulator



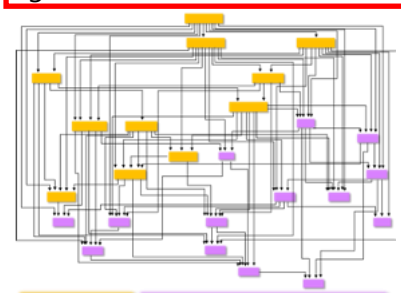
Glycoside hydrolase genes

It is necessary to identify and utilize a regulator for control of specific genes

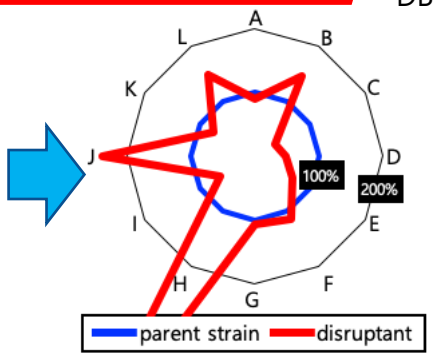
Utilization of smart cell technology for gene regulatory network construction



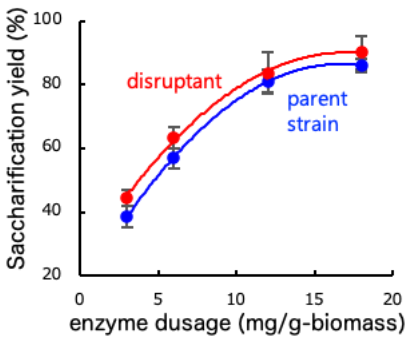
DBTL cycle for strain development



Regulatory network of glycoside hydrolase genes



Modification of enzyme ratio



Enhancement of biomass saccharification

Commercialization Plan

- By establishing the technology to change the enzyme composition in this project, we will consider providing enzymes with high cost performance to companies aiming for biomass refinery.

References

- Ogasawara, W. (2018). Technology development for simultaneous production control of multiple enzymes of cellulase-producing filamentous fungi. In: Kuhara S. (ed) Smart cell industry. CMC Publishing, PP. 156-163.

Contact Information

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Enzyme: Kazuaki Igarashi, Kao Corporation

E-mail: igarashi.kazuaki@kao.com **URL:**

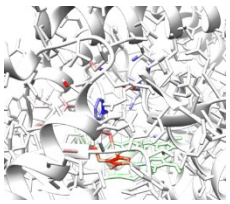
Validity verification of technology for enhancing enzyme activity:
Development of a mutation site ranking system for enzymatic function

KNC Laboratories Co., Ltd., AIST

Case Description

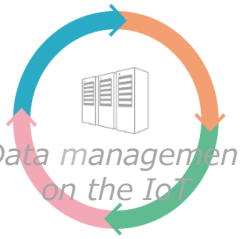
Regioselectivity and productivity can become issues when using enzymatic reactions. To solve regioseletivity issues, we developed a new mutation site ranking system for regioselectivity control of the enzyme using molecular dynamics simulation data which proposes amino acid residues that enhance the regioselectivity of enzymatic reactions.

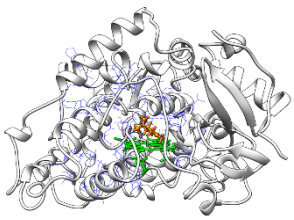
Using this method, the production ratio of the mutant enzyme increased to 48% compared to 8% for the wild type, and the amount of product increased to 1.5 times the previous amount.



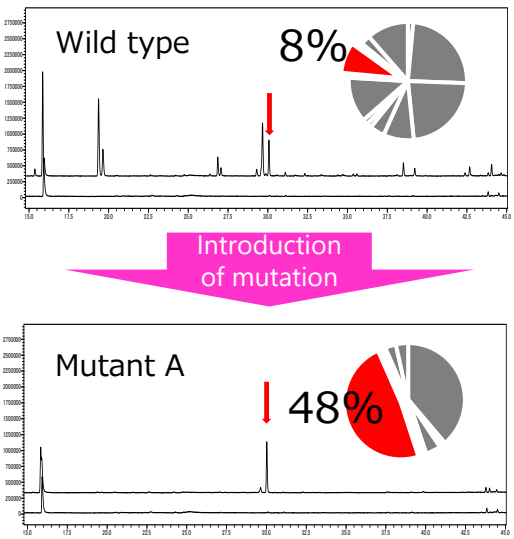
Propose modified site based on docking structure contact information

Suggest mutation site using MD simulation analysis





Develop docking structure analysis method using MD simulation



Functional analysis of enzymatic activity

Commercialization Plan

- Contract research and manufacturing for enzymes, etc.

References

- Molecular dynamics simulation:

Ikebe, J., *et.al.*, *J. Comput. Chem.*, 35(1), 39-50 (2014)

Ikebe, J., *et.al.*, *Biophys. Rev.*, 8, 1-8 (2016)

Contact Information

KNC Laboratories Co., Ltd.

E-mail: m_suzuki@kncweb.co.jp **URL:** http://www.kncweb.co.jp/en

Production of useful isoprenoids:

Improving expression of key enzymes in the isoprenoid pathway

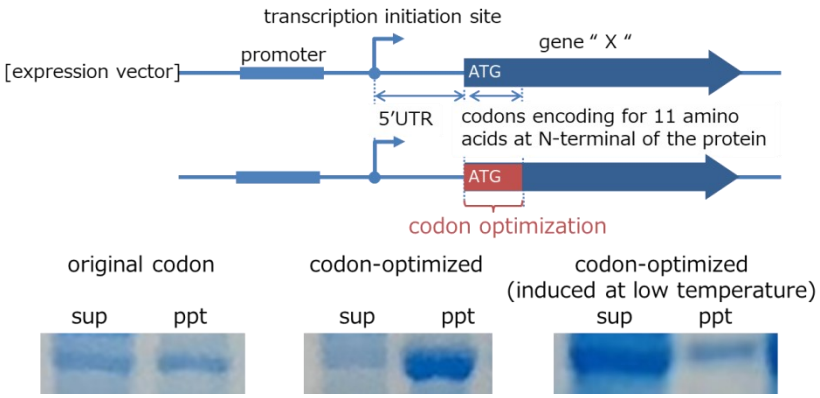
Mitsubishi Chemical Corporation, JSR Corporation, Advanced Industrial Science and Technology, Kobe University, Kyoto University

Case Description

We have been developing microorganisms which produce industrially valuable isoprenoids fermentatively. We employed gene "X" encoding for the key enzyme from heterologous microorganisms in order to alleviate the bottleneck of the isoprenoid pathway. However, the expression level of gene "X" was quite low. We then attempted to design the DNA sequence of the gene using the codon optimization technology (Sci Rep. 2019; 9:8338), so that we could obtain a higher expression level.

The synonymous substitutions were introduced into the DNA sequence encoding for eleven amino acids at N-terminal of the protein, considering the mRNA folding energy (delta G_{UH}) at 5′ regions as well as the codon adaptation index (CAI).

As a result, the protein expression of gene "X" with codon optimization improved 4.2-fold. However, the amount of the enzyme in the soluble fraction appeared to account for 4.5 % of the total amount of the enzyme. Therefore, we examined the culture conditions and succeeded in increasing the percentage of the enzyme expressed in the soluble fraction to 36% of the total expression level (8 times that of the normal conditions) by inducing the gene expression at a low temperature.



Commercialization Plan

- Aim: Develop business of fermentatively produced isoprenoids and their derivatives
- Plan: 1) Establish the methods of producing isoprenoids fermentatively and derivatizing them to useful chemicals at a laboratory scale. 2) Estimate the manufacturing cost and quality of the products through use tests. 3) Design the manufacture processes of the products considering the cost and the quality of products at a pilot scale.

References

- Sci Rep. 2019; 9: 8338.

Contact Information

Mitsubishi Chemical Corporation Science & Innovation Center Biotechnology Laboratory
Takeshi Sakamoto
E-mail: sakamoto.takeshi.mw@m-chemical.co.jp **URL:** https://www.m-chemical.co.jp/

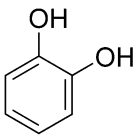
Improving productivity of useful aromatic compounds by *Corynebacterium glutamicum*:

Productivity improvement by utilizing the smart cell design systems

Research Institute of Innovative Technology for the Earth (RITE)

Case Description

The target chemical in this project



Catechol

Raw material for pharmaceuticals and fragrances
Versatile chemical whose market is expanding

Challenges for microbial production

- Toxic to most microorganisms
- Sugar-based production involves long and complex pathways

→ High concentration production was not attainable by fermentation.

By applying smart cell design systems on *Corynebacterium glutamicum* that is highly resistant to various compounds, we aim to produce high-concentration catechol.

Collaboration core technologies

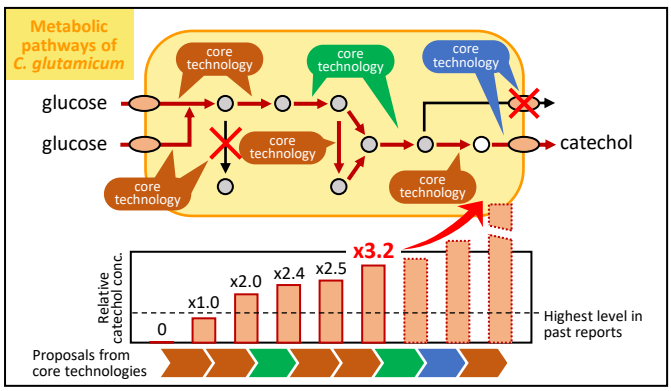
Core technologies already applied

- Technology for metabolic pathway designs
- Technology to increase protein expression level
- Quantitative targeted proteomics

Core technologies under verification

- Platform for screening of industrially beneficial transporters
- Gene Regulatory Network Modelling
- Knowledge extraction and machine learning
- Technology for enhancing enzyme activity
- Improved transcriptomics
- High-precision metabolome analysis technology

Achievements



Productivity was improved step-wisely by adopting recombination proposals from core technologies. In the first few years, our production has exceeded the highest concentration of past reports.

Commercialization Plan

Further strain development is ongoing to improve productivity to commercial-level. Currently, catechol is manufactured from petroleum-derived raw materials. We aim to establish the fermentation technology of this aromatic compound using renewable resources, thus creating and realizing new industries known as Green Bio Business.

References

- Inui, M. Development of green chemical production technology to realize a low carbon society. *BioPla Journal* **17**:15-19. 2018.
- Inui, M. Development of biofuel & green chemical production technologies to realize a low carbon society. *Society of Biomass Utilization* **19:**25-34. 2018.
- Kitade, H. and Inui, M. Development of bioproduction technology for aromatic compounds. *Japan Plastics* **107**:20-23. 2019.
- Toyoda, K. and Inui, M. Development of biorefinery technology for bioeconomy. *Journal of environmental conservation engineering* **48**:141-145. 2019.

Contact Information

Research Institute of Innovative Technology for the Earth (RITE)

E-mail: mmg-lab@rite.or.jp **URL:** http://www.rite.or.jp/bio/

Improvement of the productivity of red Monascus pigment:

An application of Gene Regulatory Network Modeling

Ezaki Glico Co., Ltd., RIKEN, Biojet Co., Ltd., AIST

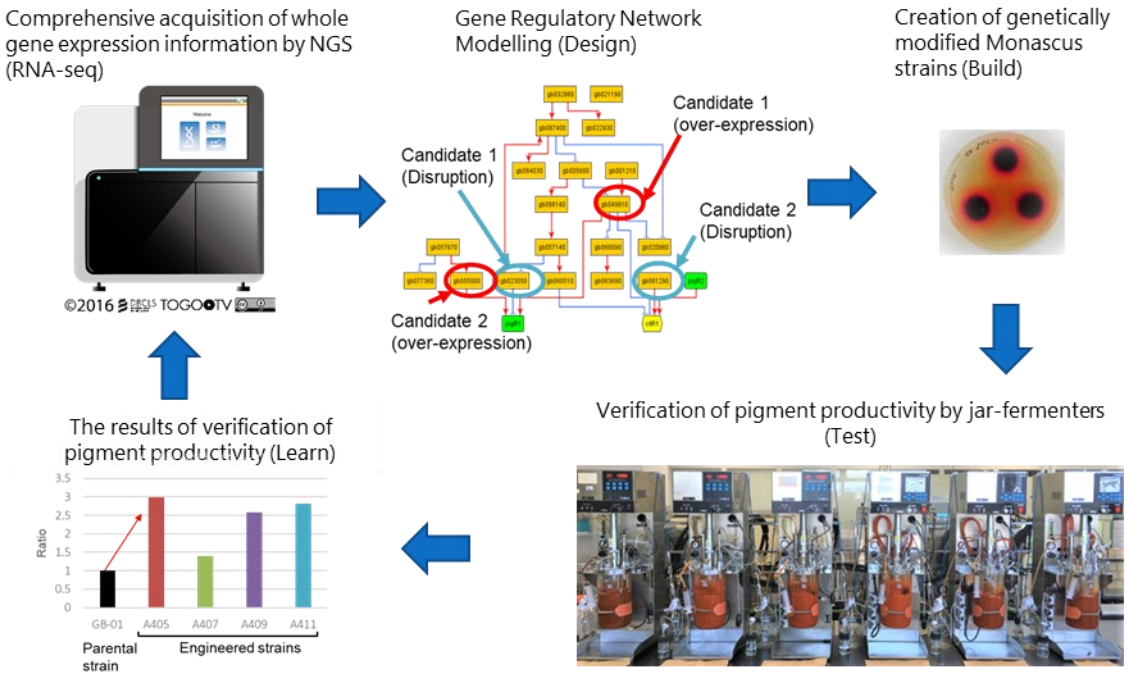
Case Description

•Red koji (Monascus purpureus) is a kind of filamentous fungi (mold).

•The extracted red pigment (also called Monascus pigment) is used in many food processing applications as a red food colorant.

•Low productivity is a problem because fungi found in nature had been used.

•Application of Gene Regulatory Network Modeling, which is one of the smart cell technologies, improved the pigment productivity by about 3 times.



Commercialization Plan

- We plan to (1) study the practical application of the productivity-enhancing strains, (2) study Monascus pigment productivity in various pilot fermenters for genetically modified organisms, and (3) deal with various safety issues and domestic and international regulations.

References

- Kumagai et al., Whole-Genome Sequence of Monascus purpureus GB-01, an Industrial Strain for Food Colorant Production. *Microbiology Resource Announcements* 8(24), 2019. DOI: 10.1128/MRA.00196-19

Contact Information

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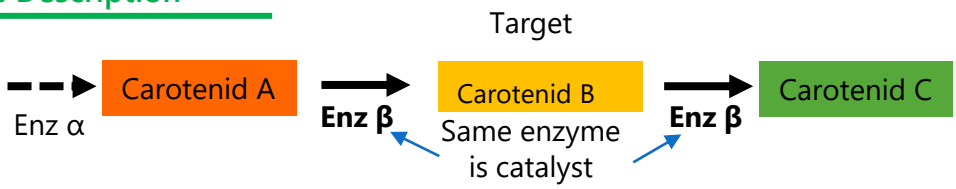
Validity inspection of a new production method for paprika-origin carotenoids using microorganisms

Ezaki Glico Co. Ltd., AIST, Ishikawa Prefectural Univ., Kyoto Univ.

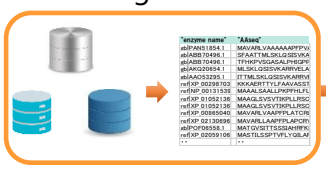
Case Description



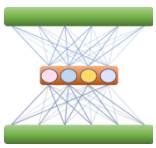
[Background] Two types of Enz β exist (accumulating and non-accumulating carotenoid B) based on species of origin

[Goal] Use dry technologies to wash out accumulated enzymes and accumulate a large amount of carotenoid B

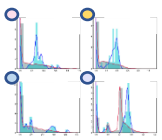
<Knowledge extraction and machine learning>



Homology search for target enzyme



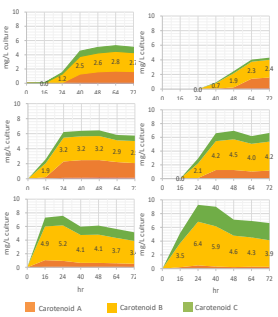
Construction of Auto Encoder model



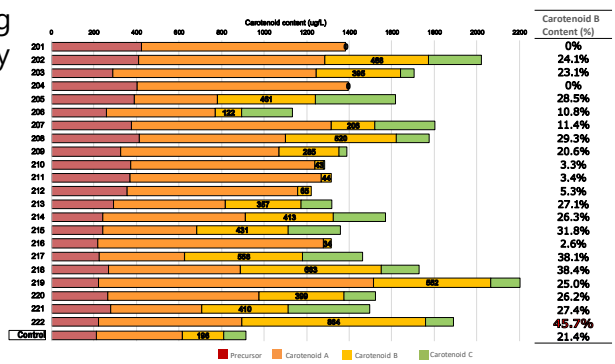
Extraction of mid-layer filter, show score distribution

Pick up mid-layer filter possessing targeted feature

Integrate candidate genes into microorganism, inspect validity under various culture conditions



[Result] After screening and specifying with dry technologies, more than 150 gene candidates were integrated into microorganisms. Carotenoid B content produced increased from 21.4% to 45.7%.



Commercialization Plan

- Increase scale of culture
- Validity inspection in practical saccharomyces species

References

- Takemura, M. et al., Applied Microbiology and Biotechnology 103: 9393 (2019)
- Araki, M. et al., Journal of Chemical Information and Modeling, 60(3), 1833-1843 (2020).

Contact Information

Ezaki Glico Co., Ltd.
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Verification of platform technology in material production

Validation by enhancement of productivity of omega-3 polyunsaturated fatty acids:

Development of yeast with high omega-3 fatty acids by computational analysis

AIST, Fuji Oil Holdings Inc, RIKEN, Nagaoka Univ. of Tech., Osaka Univ., Kyoto Univ., Kyushu Univ., NUPALS

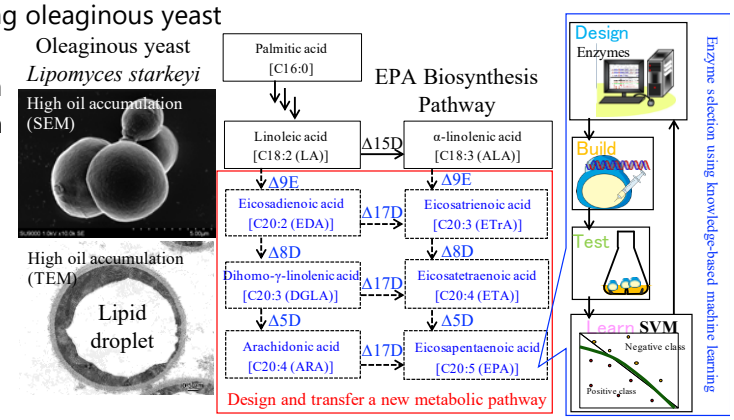
Verification of platform technology in material production

Case Description

Development of oleaginous yeast for the high production of high-added value omega-3 polyunsaturated fatty acids

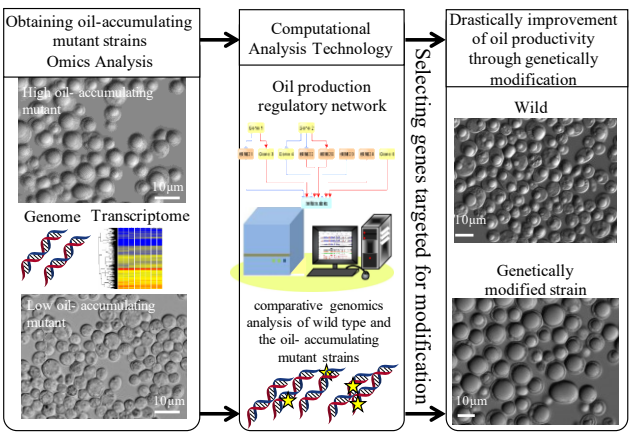
■ Development of new EPA-producing oleaginous yeast

Through design and introduction of a new metabolic pathway and selection of EPA synthesis enzymes using knowledge-based machine learning, we succeeded to develop a new oleaginous yeast with oils containing EPA (right figure).



■ Drastically improved oil productivity in genetically modified oleaginous yeast

Through comparative genomics analysis of the wild type and oil-accumulating mutant strains and selection of genes targeted for modification using regulatory network analysis with transcriptome data, we succeeded in drastically improving the oil productivity of oleaginous yeast (right figure).



Commercialization Plan

- We will further utilize data-driven smart cell technology (DBTL workflow) and work to improve oil productivity towards implementation

References

- Japanese unexamined patent application 2019-146543
- Journal of Physics: Conf. Ser., 1391 (1), 012043 (2019)

Contact Information

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Verification of the effectiveness of new production methods for alkaloids and other substances using microorganisms:
Production of plant alkaloids by microbial fermentation

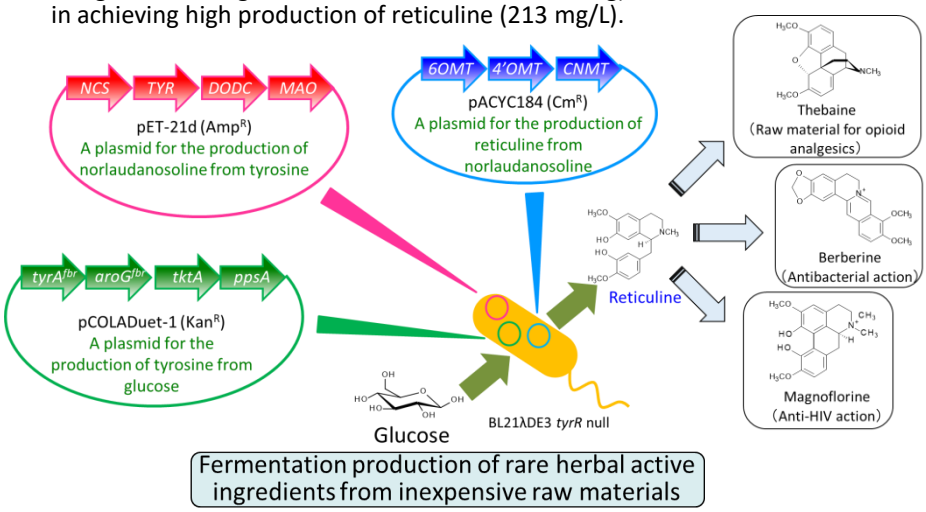
Ishikawa Prefectural University

Case Description

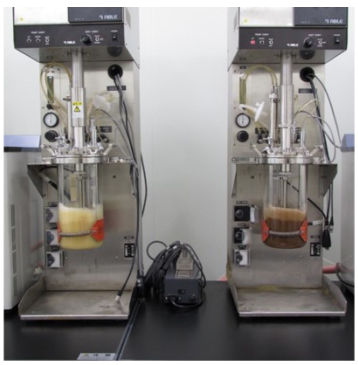
Alkaloids have various biologically active properties and are effective as seeds for new highly functional products. However, the content of alkaloids in plants is low and most of them have not been commercialized.

Microbial production using synthetic biology has been studied, but due to the metabolic pathways that span more than 10 stages, it is difficult to put them to practical use by simply examining gene combinations and culture conditions.

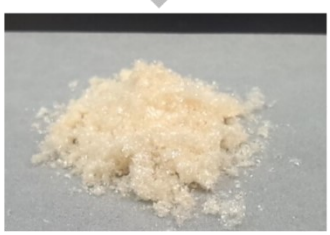
By introducing a new metabolic pathway designed using the development of information analysis technology (technology for metabolic pathway designs, knowledge extraction and machine learning), we have succeeded in achieving high production of reticuline (213 mg/L).



Fermentation production of rare herbal active ingredients from inexpensive raw materials



Alkaloid production by jar fermenter



Crude product of reticuline

Commercialization Plan

- Microbial production of thebaine (a raw material for painkillers) on the *E. coli* platform
- Development of pharmaceutical ingredients and functional foods and cosmetics for health promotion by efficiently producing alkaloids.

References

•METHOD FOR PRODUCING PLANT BENZYLISOQUINOLINE ALKALOID, WO/2012/039438, PCT/JP2011/071520
•Mechanism-based tuning of insect 3,4-dihydroxyphenylacetaldehyde synthase for synthetic bioproduction of benzylisoquinoline alkaloids. *Nature Communications* 10, 2015 (2019).
•Microbial production of novel sulphated alkaloids for drug discovery. *Scientific Reports* 8, 7980 (2018).

Contact Information

Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University
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URL: https://www.ishikawa-pu.ac.jp/research/research_institute/

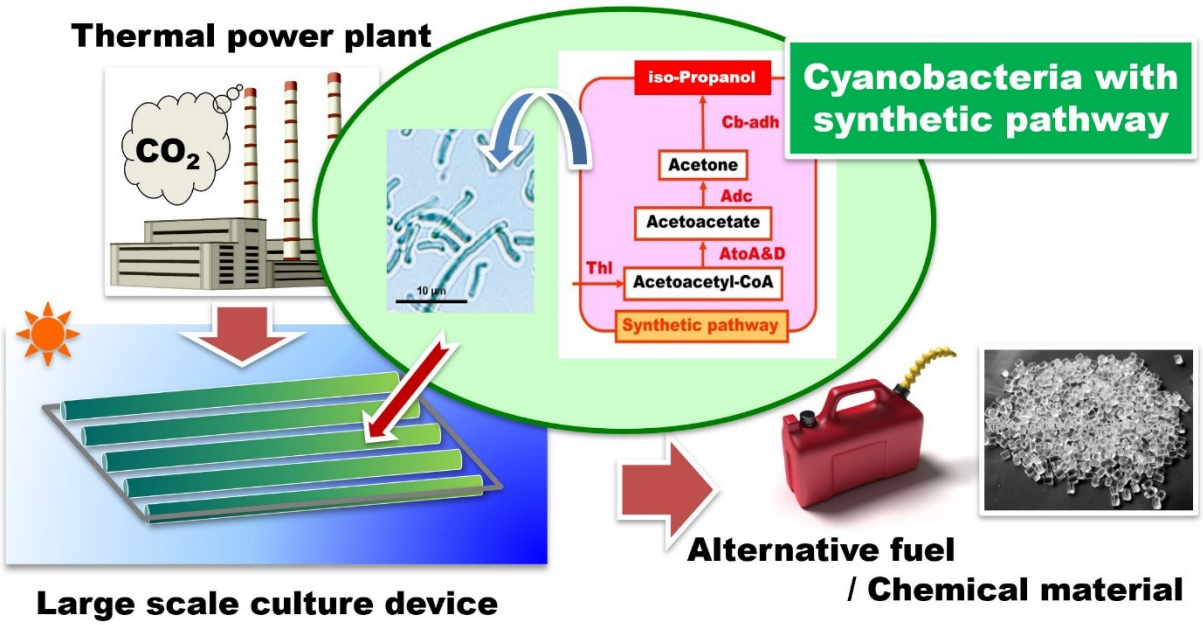
Chemical production by cyanobacteria:

Direct production of chemicals from CO_2

Kyushu University

Case Description

Our group studies the production of alternative fuel and/or chemicals using microorganisms known as cyanobacteria, which can grow on carbon dioxide and light energy. We have successfully produced isopropanol, lactic acid, 1,3-propanediol, and glycerol directly from carbon dioxide using genetically engineered cyanobacteria with a synthetic metabolic pathway consisting of multiple enzyme genes.



Commercialization Plan

- Direct production of target chemicals from carbon dioxide using cyanobacteria

References

- Hirokawa, Kubo, Soma, Saruta, Hanai, Metabolic Engineering, 57,23-30 (2019).
- Hirokawa, Maki, Hanai, Metabolic Engineering, 39, 192-199 (2017).
- Hirokawa, Maki, Tatsuke, Hanai, Metabolic Engineering, 34, 97-103 (2016).

Contact Information

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E-mail: taizo@brs.kyushu-u.ac.jp **URL:** http://www.brs.kyushu-u.ac.jp/~taizo/

High-performance plastic derived from plants:
Development of fermentation technology for polyamide precursor production

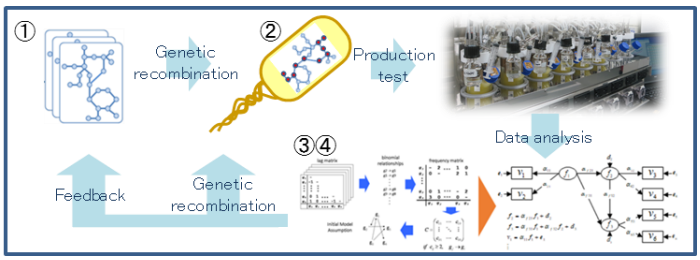
Toray Industries, Inc., AIST, RIKEN

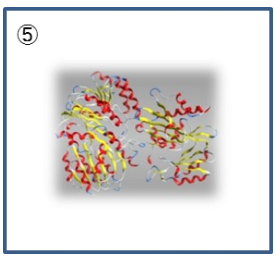
Case Description

【Purpose】

- Produce raw materials for high-performance plastic (polyamide) derived from plant biomass.
- Achieve high-level production of polyamide precursor by microbial fermentation process.

【R&D outline】



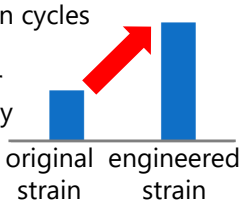


① Metabolic simulation (RIKEN)
② Strain engineering (Toray)
③ Inference of transcriptional network (AIST)
④ Gene expression level tuning (AIST)
⑤ Structure-based enzyme engineering (AIST/Toray)

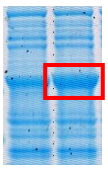
【Results】

①②
Aim: Optimize biosynthesis
- Strain engineering based on *in silico* prediction and verification cycles

2.4x higher productivity



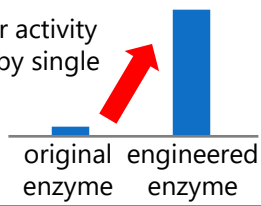
④
Aim: Strengthen limiting step
- Nucleotide sequence design to enhance gene expression



Increased expression of target enzyme

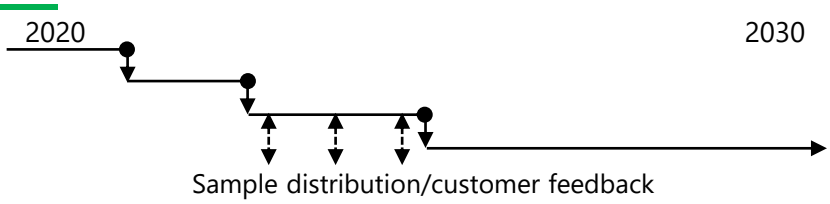
⑤
Aim: Enhance enzyme activity
- Enzyme engineering based on improved MD simulation

14x higher activity achieved by single mutation



Commercialization Plan

Lab-scale method development
Bench-scale test
Pilot-scale test
Mass production



References

- Y. Saito et al., Scientific Reports, 06 Jun 2019, 9(1):8338
- J. Ikebe et al., Journal of Computational Chemistry, 26 Oct 2013, 35(1):39-50

Contact Information

Kyohei Isobe, Toray Industries, Inc.
E-mail: kyohei.isobe.f3@mail.toray **URL:** https://www.toray.co.jp/

Production of diagnostic enzymes:
Development of smart cells for cholesterol esterase

Asahi Kasei Pharma Corporation, National Institute of Advanced Industrial Science and Technology

Verification of platform technology in material production

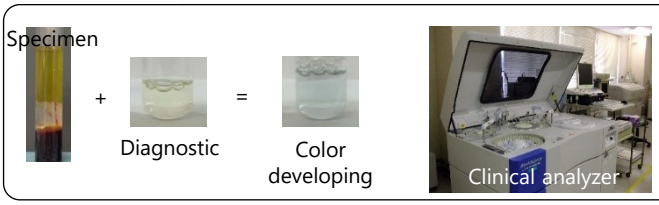
Case Description

B. stabilis cholesterol esterase, for which demand is increasing

Cholesterol esterase from B. stabilis

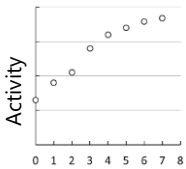
material

Increasing demand

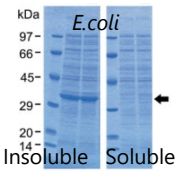


Measurement of total cholesterol, LDL-C, HDL-C, etc. using diagnostics and clinical analyzer

Limitations of conventional technologies



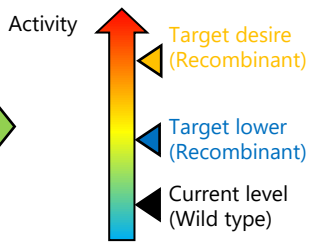
Breeding saturation



Insolubilized recombinant cholesterol esterase

Recombinant *B. stabilis*

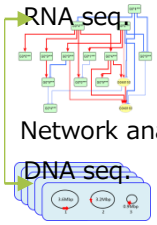
Lysed host with cholesterol esterase



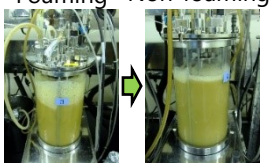
Development of B. stabilis smart cells

Development of *B. stabilis* smart cell host

B. stabilis



Mutant library analysis



Development of practical culture methods

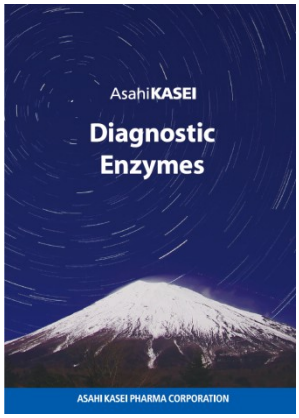
Increasing the scale of the developed manufacturing method

Commercialization Plan

- We plan to manufacture and sell cholesterol esterase.

References

- Patent application number 2018-103382,
- Patent application number 2020-027721
- K. Konishi *et al.* Genome Announc. 2017 Jul 20;5(29):
- K Yoshida *et al.* Biosci Biotechnol Biochem. 2019 Oct;83(10):1974-1984.



Contact Information

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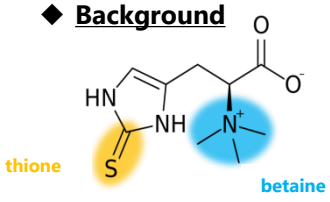
E-mail: sakasegawa.sb@om.asahi-kasei.co.jp **URL:** https://www.asahikasei-pharma.co.jp/

Development of smart cells for high production of the rare amino acid ergothioneine

NAGASE & CO., LTD.
National Institute of Advanced Industrial Science and Technology, Kobe Univ., Nara Institute of Science and Technology, Tohoku Univ.

Case Description

◆ **Background**

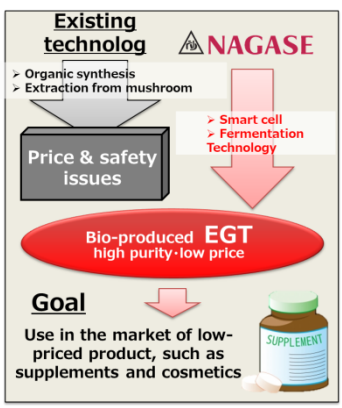


L-Ergothioneine (EGT)

- ✓ Ergothioneine(EGT) is a natural rare amino acid produced only by some mushrooms and microorganisms.
- ✓ EGT has strong antioxidant activity. Furthermore, an inducing effect of neurogenesis has been found. So, it is thought as a vitamin-like compound.(1), (2)
- ✓ Expected to be used in food, cosmetics, pharmaceuticals, and other markets, research on their use is progressing in various fields. Although the potential of EGT is high, it is difficult to expand the market in low-price areas such as supplements because of manufacturing cost and supply stability issues.

◆ **Goal**

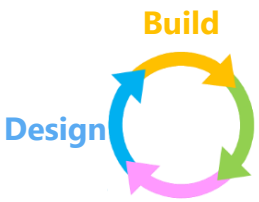
Development of a low-cost and sustainable EGT fermentation process using smart cell technology.



◆ **Focus areas**

1. **Enhancing enzyme reaction efficiency**
2. **Improvement of EGT exporting capacity**

◆ **Research flow**



D: Enhance the efficiency of enzyme reaction & predicting enzyme mutants with high activity by simulation (AIST)

B: High-throughput microorganism construction technology (NAIST, Kobe Univ.)

T: High-throughput microorganism evaluation technology (Kobe Univ., Tohoku Univ., NAGASE & CO., LTD.)

L: Extracting features of enzymes and genes from test data, which contribute to the improvement of productivity (AIST, NAGASE & CO., LTD.)

◆ **Achievements**

- ✓ Creation of high-performance enzymes was realized using enzymatic simulation.
- ✓ Original high-throughput system can evaluate thousands of cell lines each week.
- ✓ Commercializable high EGT-producing strain was established under this project.

Commercialization Plan

For commercialization of EGT, we are increasing our industrial production scale using the high EGT-producing strain and our original EGT purification process with high purity.

References

(1) I. K. Cheah, Ergothioneine; antioxidant potential, physiological function and role in disease. Biochimica et Biophysica Acta, 1822(2012), pp. 784-793
(2) B. N. Ames, Prolonging healthy aging: longevity vitamins and proteins. Proc Natl Acad Sci U S A, 115(2018), pp. 10836-10844

Contact Information

If you are interested in ergothioneine, please contact us.

NAGASE & CO., LTD.
E-mail: takeshi.nakatani@nagase.co.jp **URL:** https://www.nagase.co.jp/

Development of soy sauce Koji mold that produce a high content of natural long-chain human ceramide by using smart cells

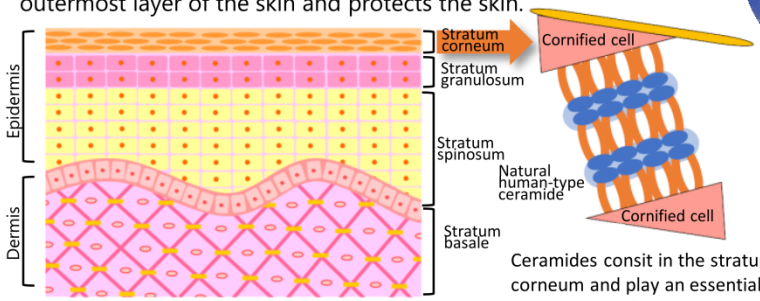
Fukuoka Soy Sauce Brewing Cooperation, Kyushu University, Kyoto University, RIKEN, AIST

Verification of platform technology in material production

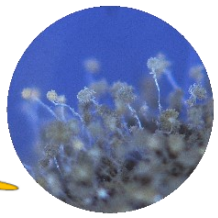
Case Description

What is ceramide?

Cermide is a type of sphingolipid that froms the outermost layer of the skin and protects the skin.



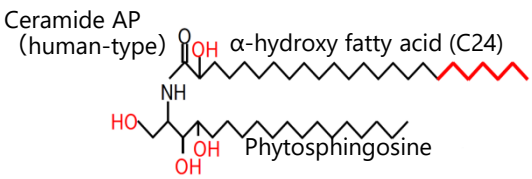
Ceramides consit in the stratum corneum and play an essential role in skin barrier function.



Koji mold for soy sauce manufacture

Aspergillus oryzae

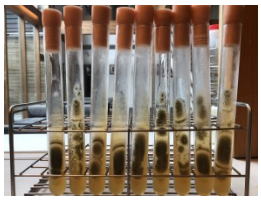
By using smart cell technology, we estimate the genes involved in the production human ceramides by *A. oryzae* and create mold strains that can produce high levels of natural human long-chain ceramides.



①Estimation of the target genes from GSM-based FBA

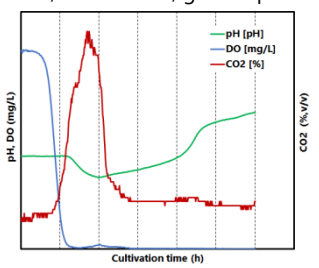
②Estimation of the target genes from gene expression control network

③Construction of recombinant *A. oryzae*

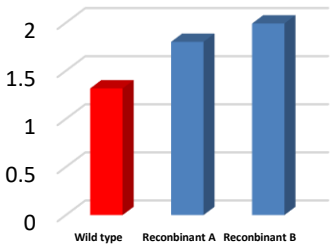


④Liquid fermentation

Cultivation data
(growth, metabolites, gene expression)



Yield of ceramides



Commercialization Plan

- Further work is planned to establish a large-scale culture system for the recombinant *A.oryzae* that can produce human long-chain ceramides.

References

- Nishide A, Shimura M, Ohnuki K, Shimizu K, Ohnuki K. *Jpn Pharmacol Ther* 2020;48:237-241

Contact Information

Fukuoka Soy Sauce Brewing Cooperation

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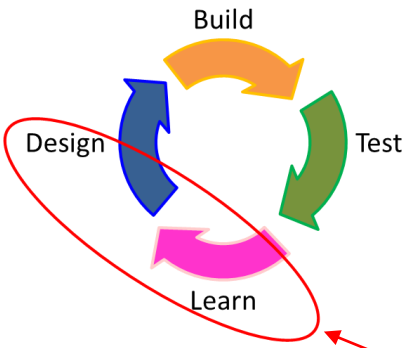
Development of industrial enzymes using molecular dynamics simulations:

Production method for valuable chemical products using enzyme design technology

Amano Enzyme Inc., National Institute of Advanced Industrial Science and Technology, Kyoto University

Case Description

Issues: In the development of industrial enzymes, "Learn" and "Design" in the BTLD cycle take time and effort, which creates a bottleneck in development.



Basic technology: MD calculation and docking simulations were used. These simulations predicted the interaction between the amino acid residues of enzymes and the specific positions of substrates.

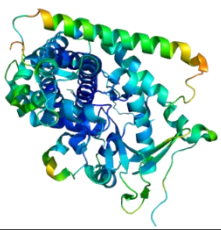
Results: Mutant enzymes were designed by predicting amino acids using MD calculation and docking simulations. To evaluate the *in silico* results, the amino acid substitution experiments were carried out. We found that several mutants showed improved properties.

Docking simulations
Molecular dynamics calculation

⇄

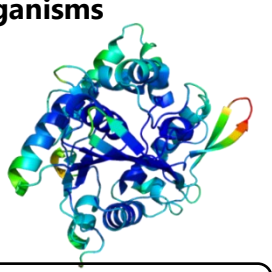
Enzyme variants

(a) Lipase from microorganisms



Improving of enzymes through enzyme design technology by MD calculation.

(b) P450 from microorganisms



Obtaining promising mutants through the BTLD cycle of enzyme design technology.

Commercialization Plan

(a) Lipase from microorganisms

We will propose outside the company methods for producing high-quality materials that have a low environmental impact using our developed enzymes.

(b) P450 from microorganisms

We will develop enzymes, which realize a position-specific oxidation reaction and contribute to the realization of a healthy society by increasing the added value of omega-3 fats and oils.

References

Patent applications in preparation

Contact Information

Dr. Satoshi Koikeda, Amano Enzyme Inc.

E-mail: satoshi_koikeda@amano-enzyme.com **URL:** https://www.amano-enzyme.co.jp/

Outreach activities:

Introduction of R&D achievements and promotion of social implementation

Japan Bioindustry Association (JBA)

Case Description

Introduction of smart cell project development technology. Please contact us if you are interested.

1. Publication of special articles on smart cell technology and case studies

- Bioscience and Industry (JBA bulletin) , 2019-2021
  Published Vol.77 No.4-6, Vol.78 No.1-4
  Future plan Vol.78 No.5-6, Vol.79 No.1-2
- Nature Published on Aug. 5, 2020, Vol. 584 No. 7819
  Focal Point on Synthetic Biology in Japan
  https://www.nature.com/collections/aehfijhibj

2. Introduction of smart cell technology via homepage and video

- Homepage

https://www.jba.or.jp/nedo_smartcell/

- Video

https://www.youtube.com/watch?v=j5x2COZSpEQ

3. Holding of technical seminars

- Abstracts of 2019 technical seminars, held 3 times
  https://www.jba.or.jp/activity/adv_biotech/rd_project/2220/
  First Second Third

4. Individual consultation

- Interviews with technical seminar participants (ongoing)

5. BioJapan2020 Exhibition

- Short presentation, exhibition of achievement, distribution of booklets etc.

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Conclusion

In recent years, there have been various developments toward the realization of a sustainable society. One significant development is the formulation of a biotech strategy for the first time in 11 years in Jun e 2019 by the government's "Council for Integrated Innovation Strategy". In January 2020, the "Progressive Environment Innovation Strategy" was formulated to solve climate change problems, which is an urgent global issue. In line with these actions, the industry, academia, and government are expected to strengthen their efforts to create progressive innovations that contribute to solving the problems from their respective standpoints.

Aiming to create a bio-economy and realize a carbon recycling society, this project has been working on technological developments that will lead to the expansion of industrial applications of material production using biological processes. We hope that the results generated from this project can be recognized by as many people as possible and can contribute to solving social issues and Japan's economic development.

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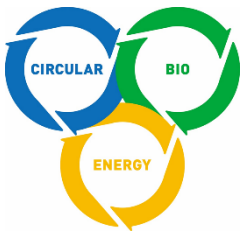
（2020.Oct.）

NEDO has defined circular economy, bio-economy, and sustainable energy as t he "three social systems that realize a sustainable society" and has established a symbol mark that expresses them. The Comprehensive R&D Principles for Sustainable Society 2020 (the NEDO's principle), which realizes the integrated and organic promotion of these three social systems and summarizes the ideal way of technological developments and the direction to aim for in order to solve the climate variability problems, has been formulated (February 2020).



3 Essential Social Systems for Sustainable Society

Implementer index (alphabetical)

索引

Implementer index (alphabetical)

索引

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