Development of Multi-Lock Biopolymers Degradable in Ocean From Non-Food Biomasses

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Implementing organizations : The University of Tokyo, Mitsubishi Chemical Corporation, Bridgestone Corporation, Teijin Limited, Kureha Corporation, Kyushu University, Nagoya University, Yamagata University, Research Institute of Innovative Technology for the Earth (RITE), National Institute of Advanced Industrial Science and Technology (AIST), Ehime University, Tokyo Institute of Technology

Research Institute of Innovative Technology
for the Earth (RITE) Masayuki INUI

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MOONSHOT RESEARCH & DEVELOPMENT PROGRAM NEDO

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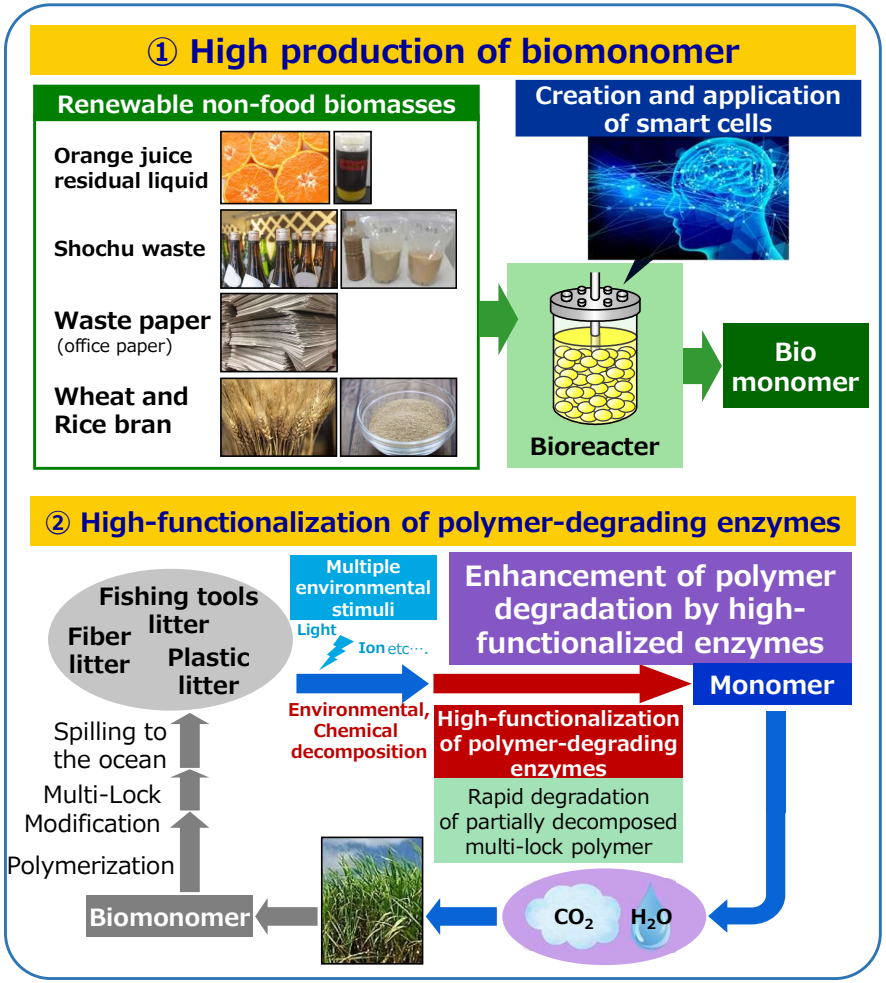
① High production of biomonomers

We designed synthetic pathways for fermentative production of biomonomers such as dicarboxylic acids demanded by several companies for polymer production. We established integrated-production of biomonomers and their precursors from glucose as the starting material.

② High-functionalization of polymer-degrading enzymes

We established heterologous expression of three polymer-degrading enzymes in *Escherichia coli* as active-forms. Furthermore, we increased activity of polymer-degrading enzymes at broad range of temperatures by means of structure-guided mutagenesis.



RITE Research Institute of Innovative Technology for the Earth

Overview of the project and our part

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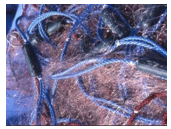
【R&D items of RITE】 ①High production of biomonomer ②High-functionalization of polymer-degrading enzymes

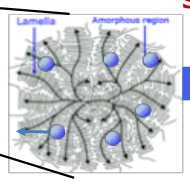
Uncollectable ocean plastic litters (high possibility of spilling into the ocean ecosystem)


Fishing tools litter


Fiber litter

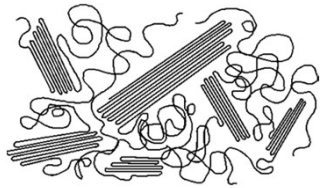

Plastic litter

Spread by accident



Multiple environmental stimuli to unlock

Light
Ion
etc….



On-demand decomposition
Environmental, chemical, and enzymatical

Enzyme  RITE

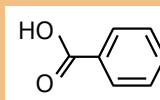
② High-functionalization of polymer-degrading enzyme

H_2O

CO_2

Photo-synthesis

E1+E3 Development of multi-lock type degrading mechanism



Bioprocess

Monomer

RITE

Non-food biomass

① High production of biomonomer

Polymerization, Processing

High durability in use

Multi-lock biopolymer

E3 Development of polymers from non-food biomass

Topic ① Bioproduction of *cis,cis*-muconic acid

Topic ② High-functionalization of PET-degrading enzymes

Matrix management of R&D, and our part

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	A Plastic Mitsubishi Chemical	B Tire Bridgestone	C Fiber Teijin, Teijin Frontier	D Fishing net Kureha	E Common theme Academia TL: Kozo ITO
E1: Multilock degradation Tokyo Univ.	●	●	●	A～D: closed theme,	E: open theme ●
E2: Evaluation of structural properties Kyusyu Univ., KIT, Kobe Univ.	●	●		●	●
E3: Synthesis･Process Nagoya Univ.,Yamagata Univ., RITE, Tokyo Inst. of Technol., Osaka City Univ., Shinshu Univ., Nagaoka Univ. of Technol.	●	●	High production of biomonomer ●	High production of biomonomer ●	High production of biomonomer Adipic acid ●
E4: Ocean degradation AIST, Ehime Univ., CERI					
E1＋E3 RITE, Tokyo Univ.			Isolation of Plastic-degrading microorganisms from ocean ●		High-functionali-zation of polymer-degrading enzymes PETase ●

Closed theme : Research and development on the bioproduction of monomers, for which Teijin and Kureha have requested bioproduction, is on going.

Open theme

R&D of polymer from non-food biomass feedstock
"High production of biomonomer"

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Renewable, unused non-food biomass

Orange juice residual liquid



··· Beverage company

Shochu waste


··· Shochu companies in Kyushu and Chugoku area

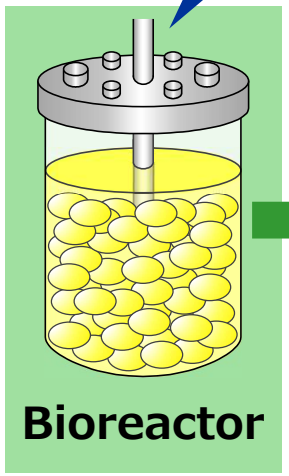
Waste paper (office paper)

··· Used paper recycling organization
··· Chemical company

Wheat and Rice bran


··· Flour and rice mill company

Creation and application of smart cells



Bioreactor

Bio-monomer

Utilization of non-food biomass (including industrial waste)
= Contribution to carbon neutral
→ Great social and economic impact (contributes to MS's Cool Earth)

Bioproduction of *cis,cis*-muconic acid, a precursor of adipic acid, from non-food biomass feedstock

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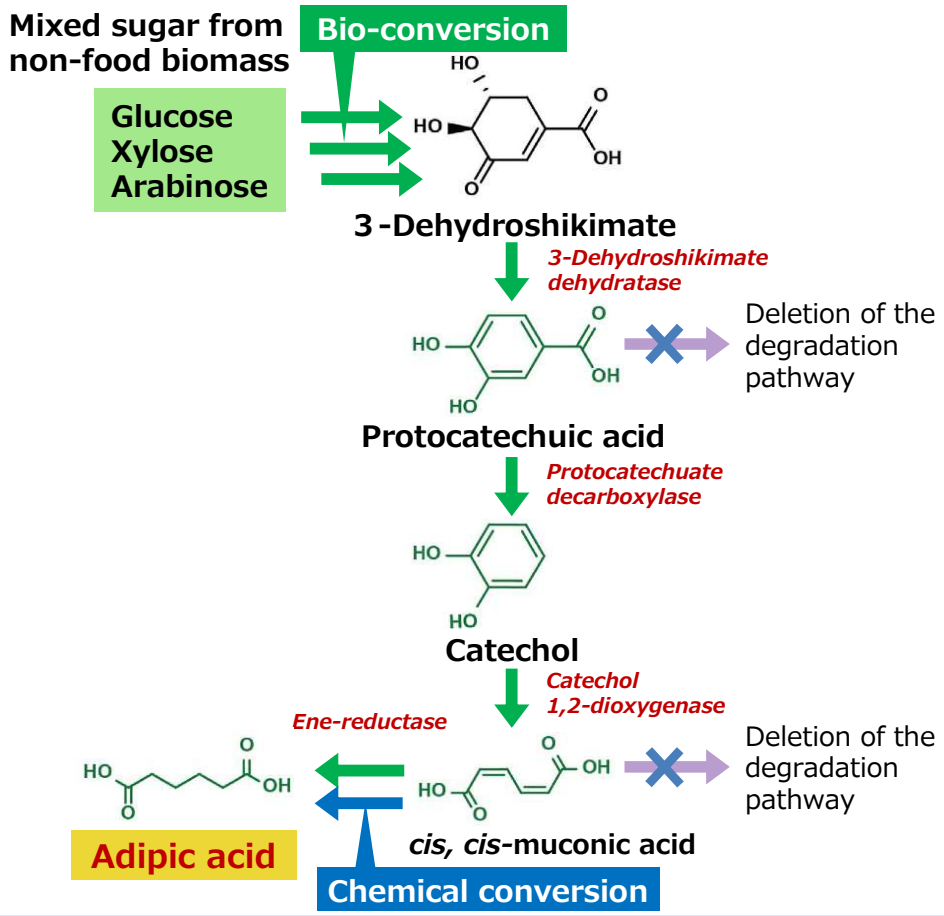
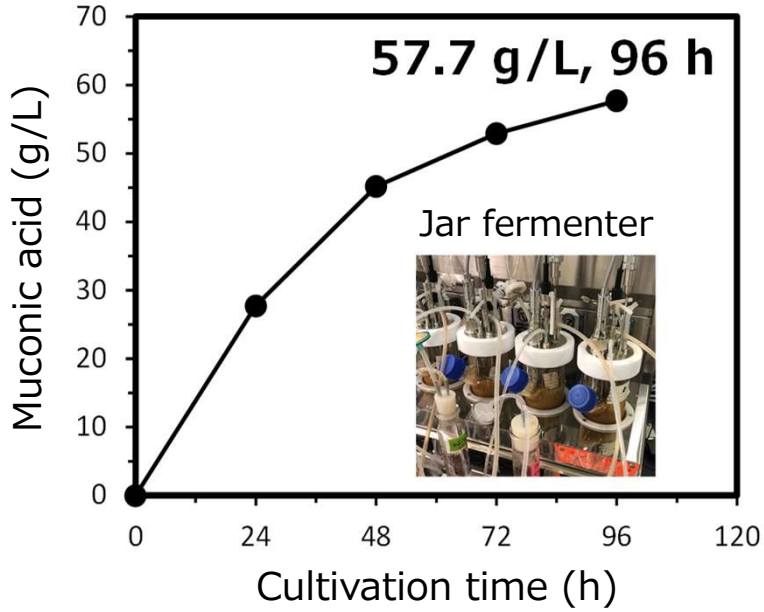
- Successful bioproduction of *cis, cis*-muconic acid, precursors of adipic acid, a raw material monomer for polyamides and polyesters (fishing tools, fibers, bottles, etc.).
- Screening of various genes for adipic aid-producing enzymes is in progress.

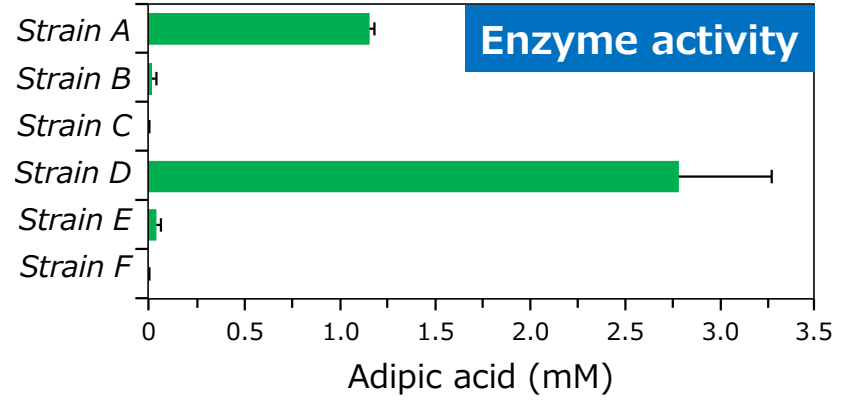
Artificial metabolic pathway for bioproduction of adipic acid



cis,cis-muconic acid production



Screening of adipic acid-producing enzymes



Comparison of bioproductivity and wide application of *cis,cis*-muconic acid

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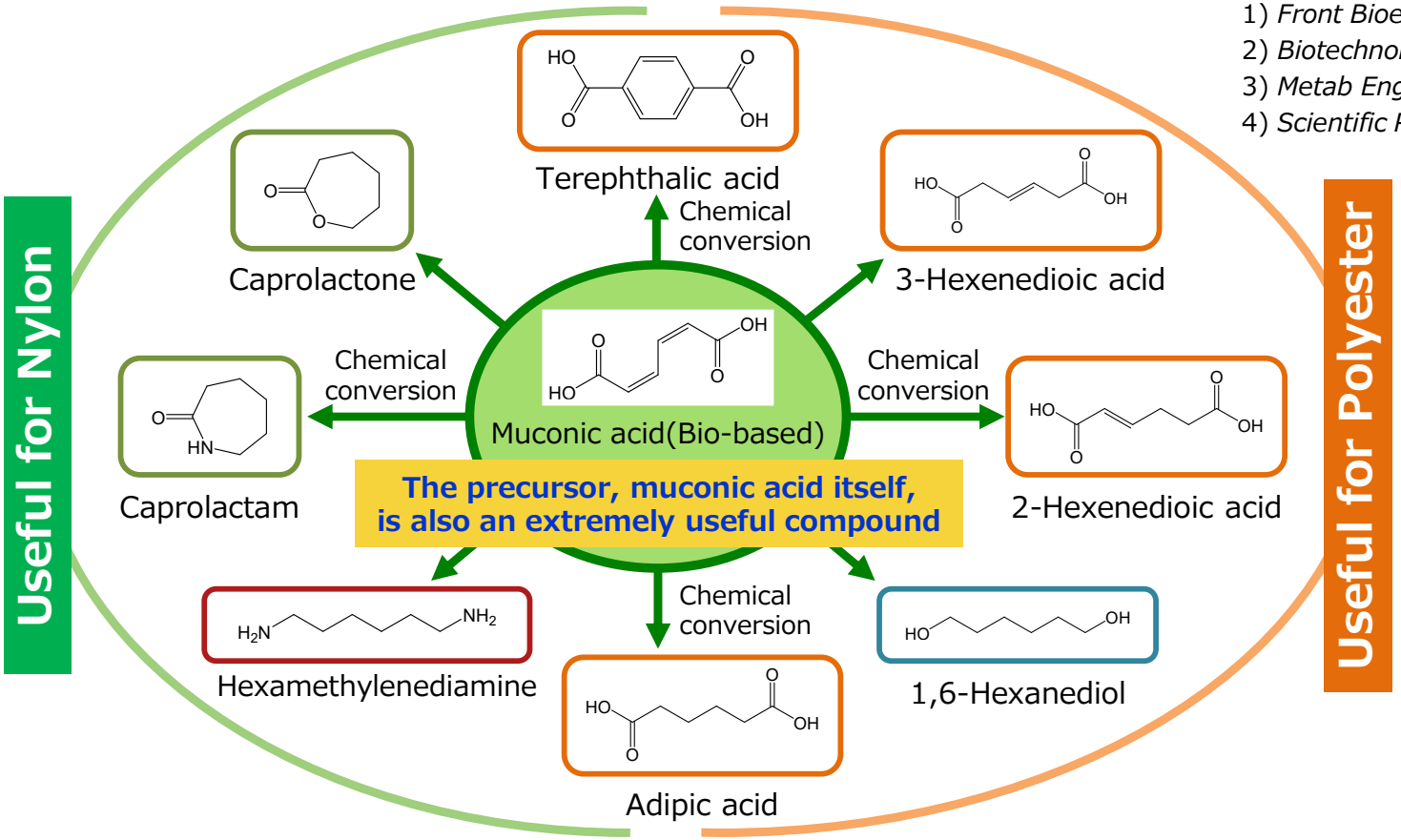
Host	Titer	Time	Research group
Escherichia coli [1)]	64.5 g/L	120 h	Choi *et al.*, 2019. (Inha University, Korea)
Escherichia coli [2)]	36.8 g/L	48 h	Niu *et al.*, 2002. (Michigan State University, USA)
Pseudomonas putida [3)]	22 g/L	104 h	Bentley *et al.*, 2020. (NREL, USA)
Corynebacterium glutamicum [4)]	54 g/L	168 h	Choi *et al.*, 2018. (Inha University, Korea)
Corynebacterium glutamicum	**57.7 g/L**	**96 h**	**RITE**



1) *Front Bioeng Biotechnol.* **9;7**:241. 2019.
2) *Biotechnol Prog.* **18(2)**:201-211. 2002.
3) *Metab Eng.* **59**:64-75. 2020.
4) *Scientific Reports,* **8**:18041. 2018.

Development of multi-lock type degrading mechanism “High-functionalization of polymer-degrading enzymes”

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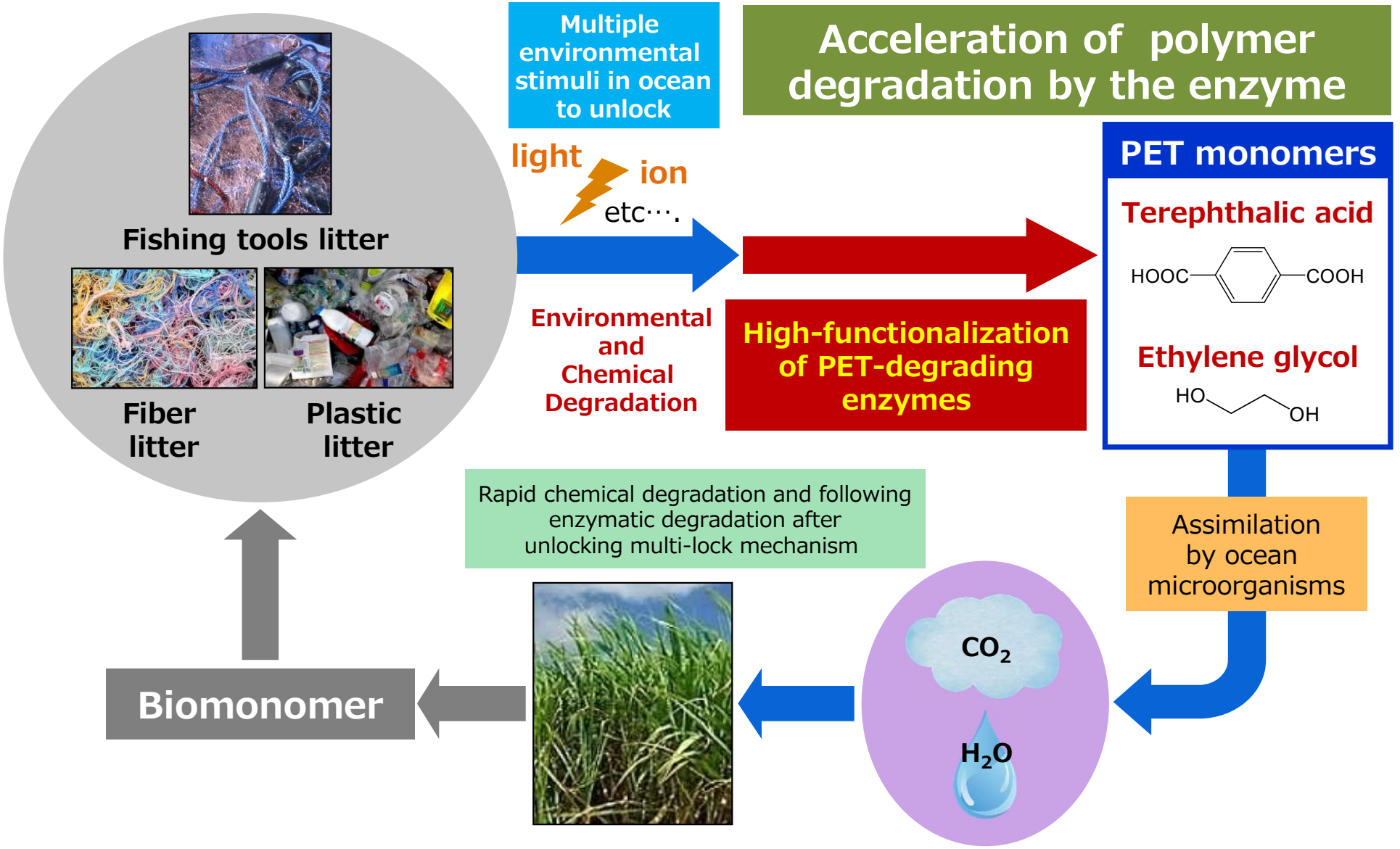

PET film degradation by PET-degrading enzymes and enhancement of the activity by mutation

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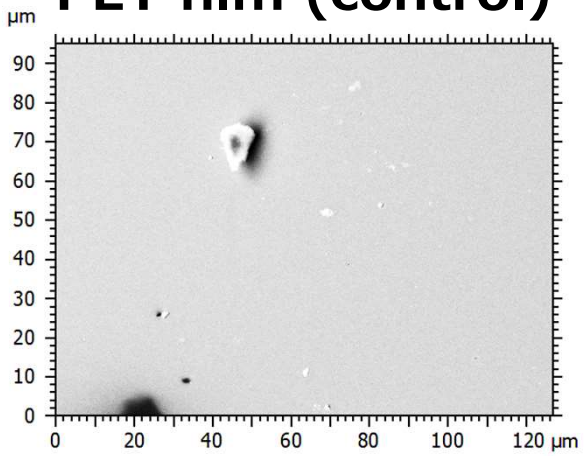
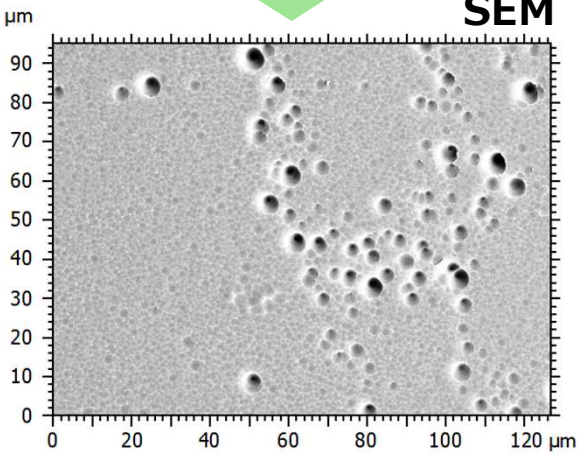
PET film (control)

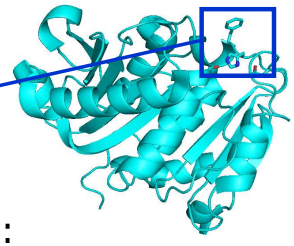


After incubation with microorganism expressing PET-degrading enzyme

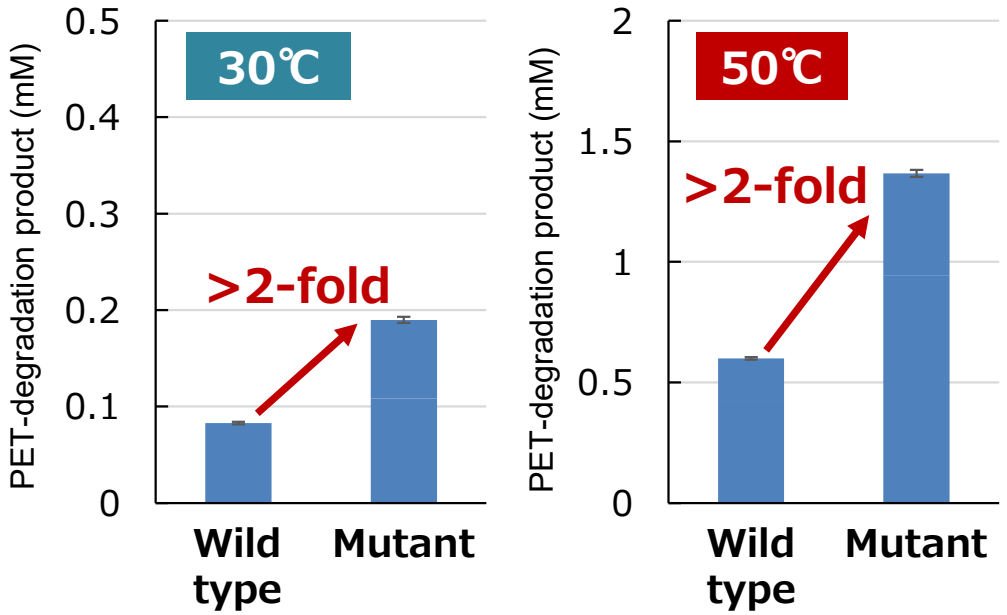
SEM



Introduction of mutation



Reaction condition:
PET powder (>40% crystallinity)
30℃ or 50℃, 23h, HPLC analysis



PET-degrading activity of the mutant increased more than 2-fold with retaining heat resistance (The mutant showed high activity in broad thermal range)

Effect of cationic surfactant on PET-degrading enzyme activity

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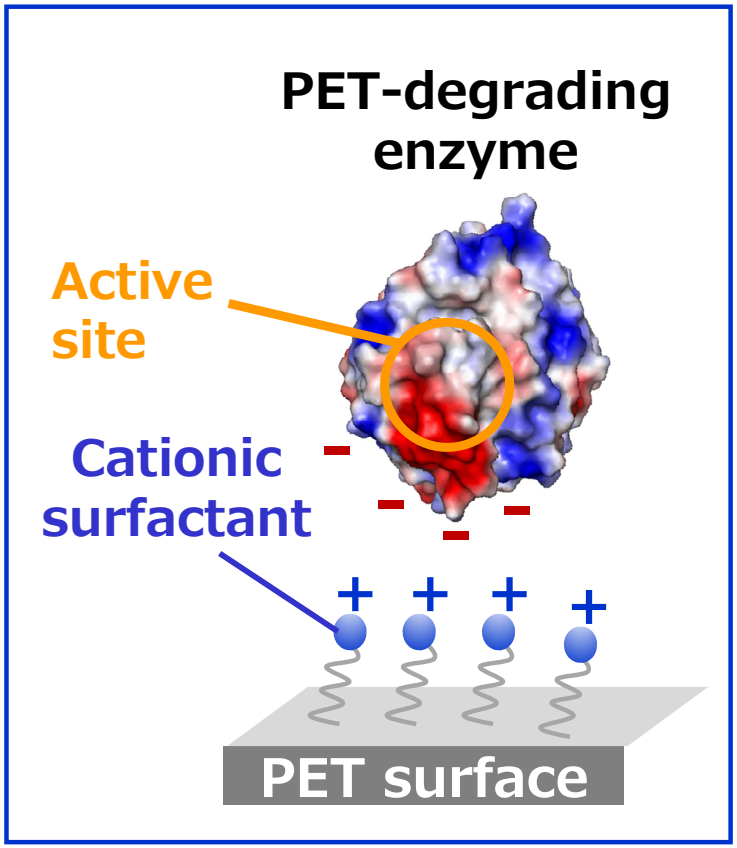

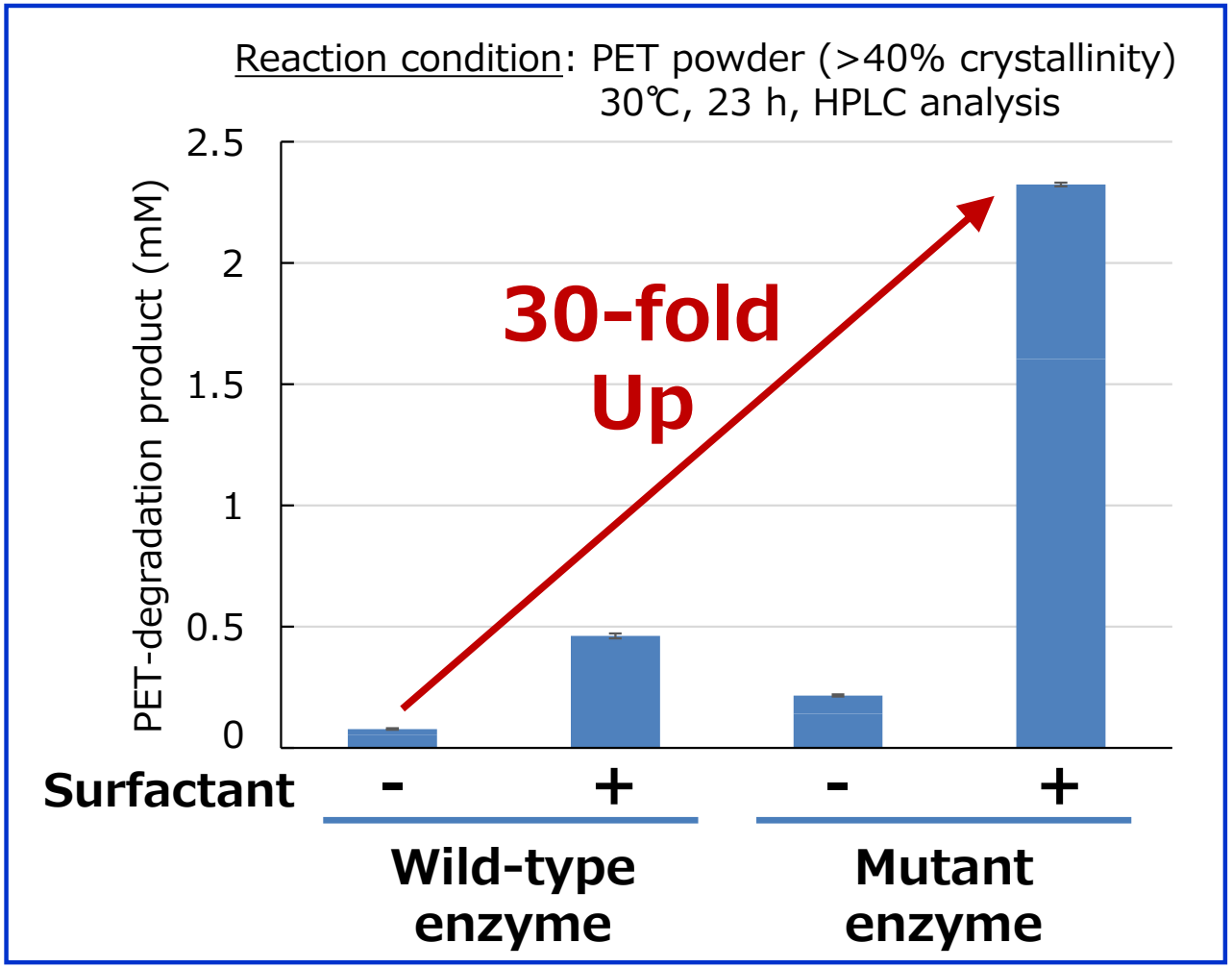

Addition of a very low concentration of cationic surfactant remarkably enhanced the enzyme activity about 30-fold comparing to the wild-type enzyme

End goal (FY2029)

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Development of Multi-Lock Biopolymers Degradable in Ocean From Non-Food Biomasses

■ High production of biomonomer

・Completion of scale-up technology for biomonomer production at pilot scale.

Construction of a strain that can produce more than 100 g/L of bio-monomers from mixed sugars derived from non-food biomass, and optimization of production conditions using a low-cost bioprocess.

■ High-functionalization of polymer-degrading enzyme

・Completion of the high production of polymer-degrading enzymes with more than 10 times higher activity at a protein concentration more than 5 times higher than the original strain at low cost on a bench scale (several kg to 10 kg).