Development of a Bioprocess That Uses Electrical Energy to Fix Atmospheric CO_2

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Implementing organizations : National Institute of Advanced Industrial Science and Technology (AIST), Tokyo Institute of Technology, Nagoya University

Ability to utilize electric energy

Enhancement of CO_2 fixation

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Providing electrotrophy （Tokyo Tech）

■Aim of the project

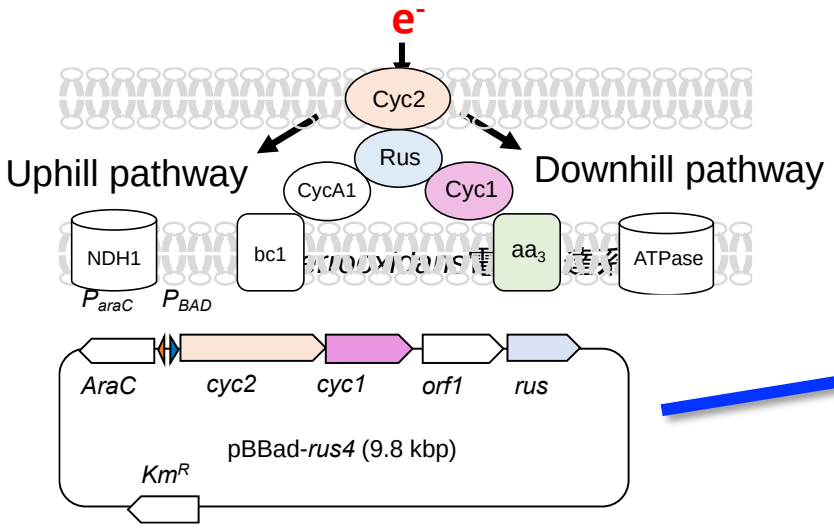
Introducing electron transfer pathway into *Ralsotnia* to provide electrotrophy

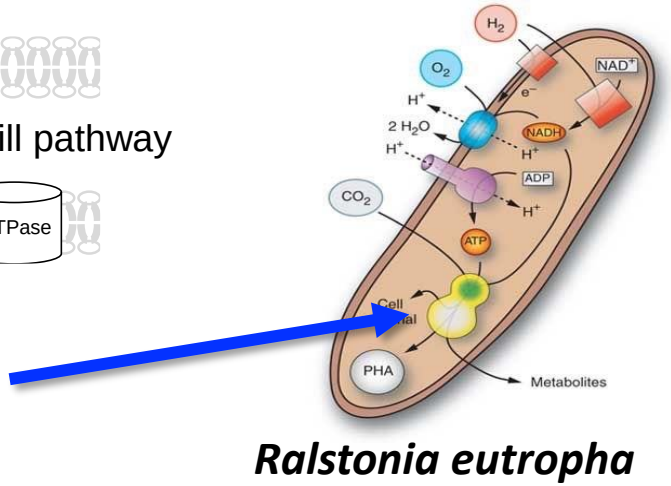
■Progress in FY2021

2-1A） Introduction of a group of electron transfer genes from *Acidithiobacillus* into *Ralstonia*

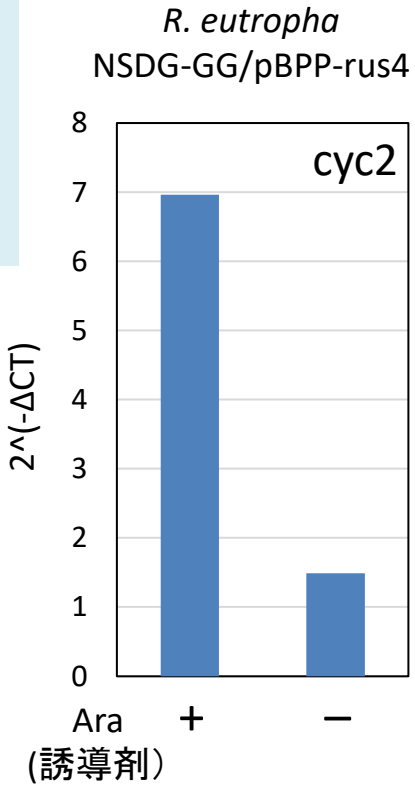
Result:

- Building and transforming inducible expression vector into *Ralsotnia*
- Confirmation of the expression of electron transfer genes using qRT-PCR





Ralstonia eutropha



Providing electrotrophy （Tokyo Tech）

■ Progress in FY2021 and future plans for FY2022

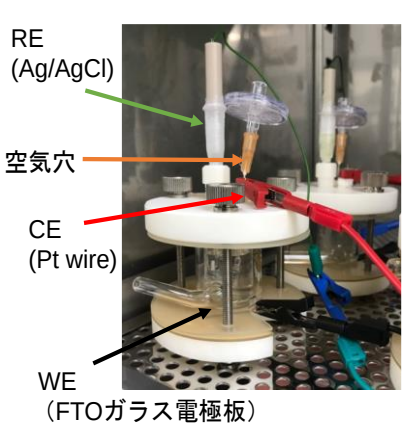
2-1B） Electrochemical measurements of *Ralstonia* strains transformed with a set of electron transfer related genes from *Acidithiobacillus*

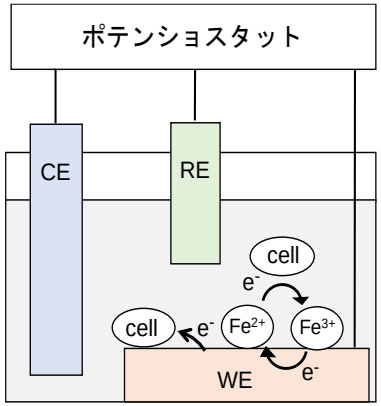
Results :

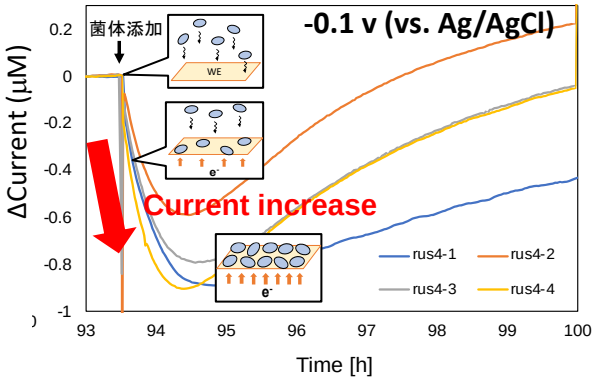
- Detection of reduction current by Downhill pathway strains
- The effect of the growth status of the fungus on the electrochemical properties is significant.

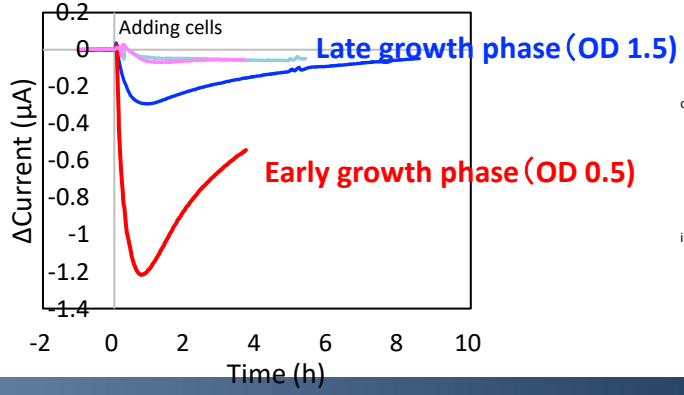
Future plans:

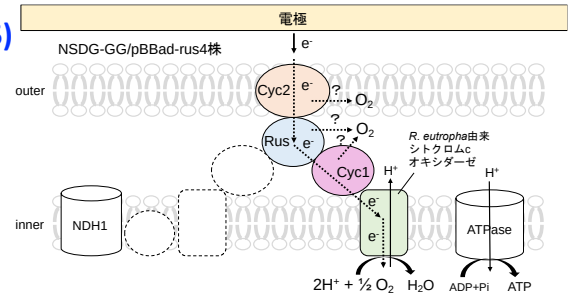
- Optimization of culture conditions and electrochemical measurement conditions
- Localization of electron transfer proteins and confirmation of ATP production
- Introduction of the Uphill pathway











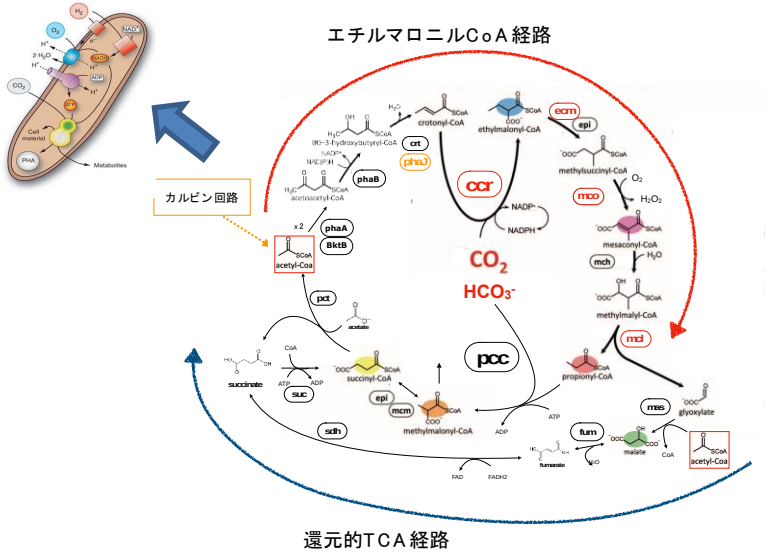
Enhancement of CO2 fixation（Tokyo Tech）

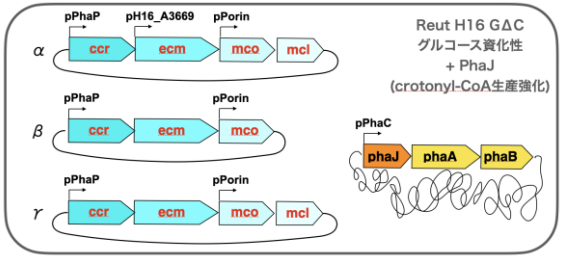
■ Aim of the project

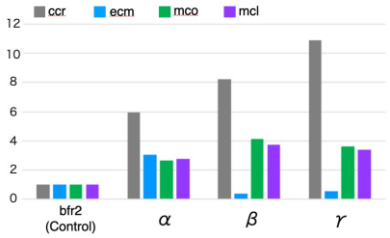
Introducing semi-synthetic CO_2 fixation pathway into Ralstonia eutropha and searching and enhancing the enzyme activity of new carbon fixation enzyme

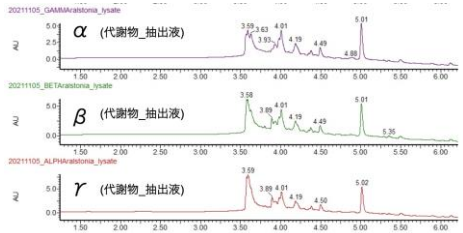
■ Progress in FY2021 and future plans for FY2022

(2-3A) Introduction of gene clusters related to the ethylmalonyl-CoA (Em-CoA) pathway, including the CO_2 immobilizing enzyme CCR, into *Ralstonia*









Results:

- Synthesis of plasmids with genes clusters for Em-CoA pathway. *Ralstonia* was transformed with each plasmid, gene expression and protein expression was confirmed.

Future plans:

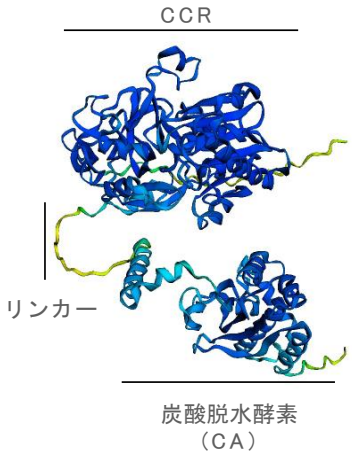
- Identification of intermediate metabolites using LC-MS
- Confirmation of CO2 fixation capacity using carbon isotopes

Enhancement of CO2 fixation（Tokyo Tech）

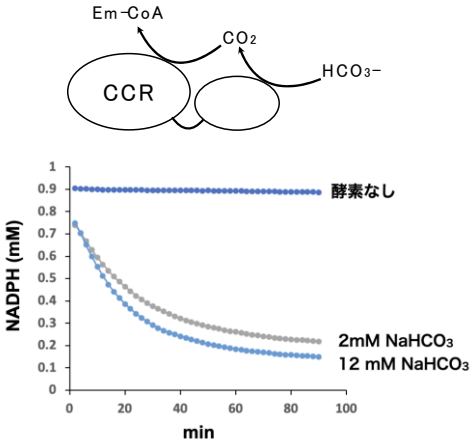
■ **Progress in FY2021 and future plans for FY2022**

2-3B) Enhancement of the enzyme activity of new identified carbon fixation enzymes

Design and synthesis of the fusion protein of crotonyl-CoA carboxylase/reductase (CCR) and carbonic acid dehydrogenase (CA) and its activity confirmed.

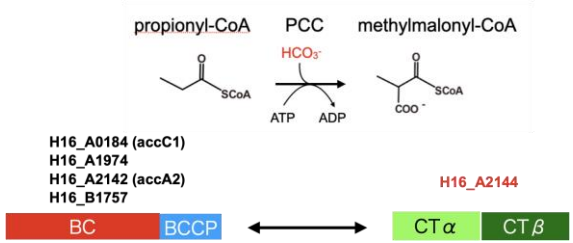


Structural prediction of CCR-CA fusion

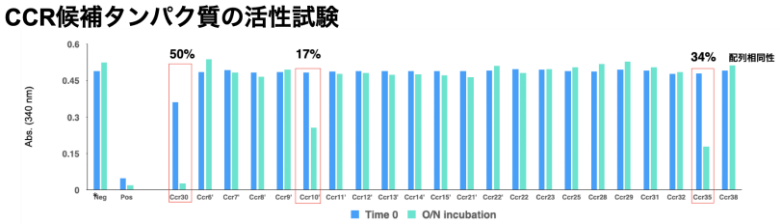


Enzyme Activity of CCR-CA fusion

Identification of Propionyl CoA carboxylase (PCC) in *Ralstonia*



Search for novel CO2-fixing enzymes in nature using machine learning



Result :

- To improve the CO2 uptake efficiency of CCR, we synthesized a fusion protein of CCR and carbonic acid dehydrogenase (CA) and confirmed its activity.
- We have predicted and confirmed functional carbon fixation enzymes in *Ralstonia* and in nature

Future plans:

- CCR-CA performance measurement under low CO_2 condition
- Isolation and purification of novelprotein candidates with PCC activity in nature
- High-throughput screening of carbon fixing enzymes with high activity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