

Screening of Photosynthetic Bacteria Utilizing Microdroplets

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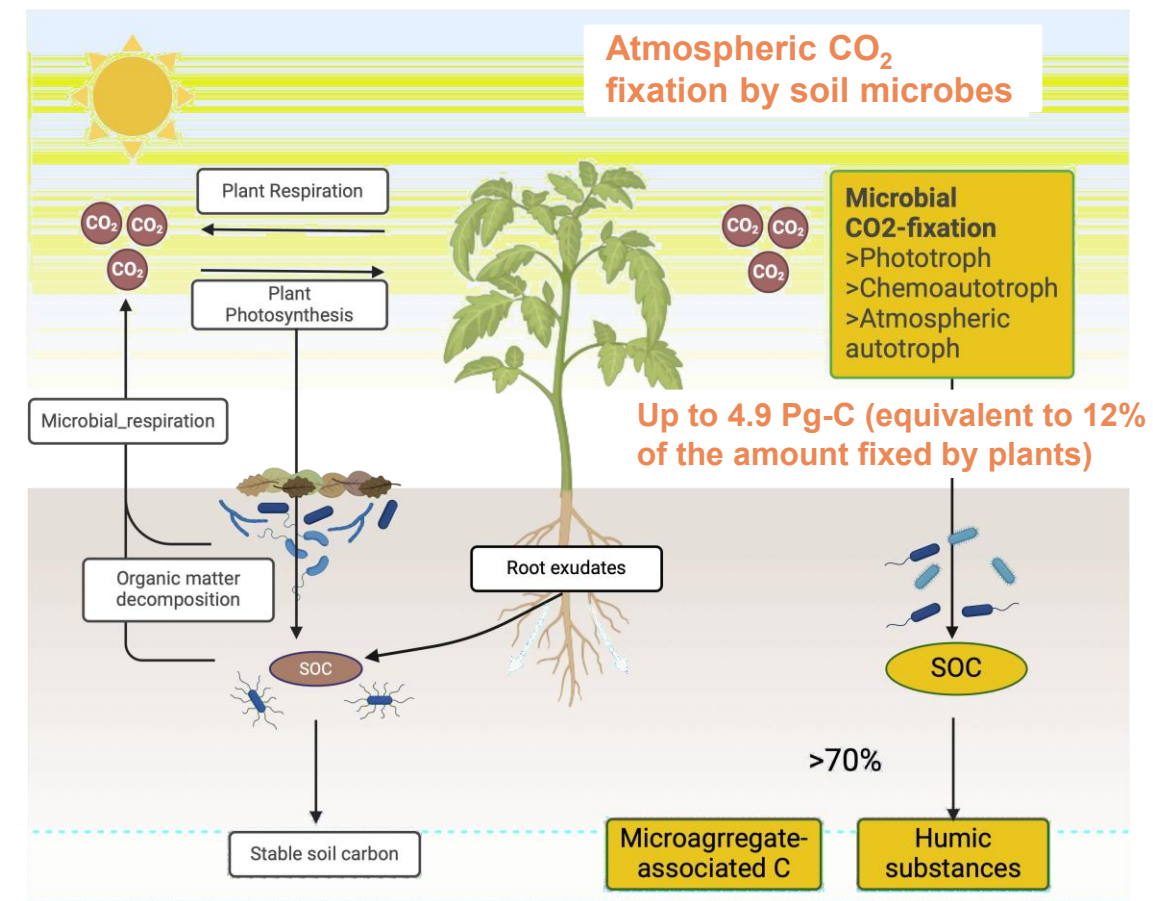
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Research Background

Traditionally, plants were considered the main contributors to atmospheric CO₂ fixation, but it has been found that soil microbes also fix CO₂ at 4.9 Pg-C per year, equivalent to about 12% of the amount fixed by plants. This fixed CO₂ becomes soil organic carbon (SOC), and more than 70% of it is stabilized as humic fractions, contributing to the maintenance of soil structure (Xiao et al., 2021). In particular, **photosynthetic bacteria** are believed to play an important role in the fixation of atmospheric CO₂ on the soil surface.

In recent years, metagenomic analysis has revealed new photosynthetic lineages at the phylum level in prokaryotes, some of which have been cultured (Yabe et al., ISME C, 2022). These findings helps us to understand that there are diverse photosynthetic lineages in the terrestrial environment that have not yet been cultured. Cultivating these uncultured photosynthetic bacteria will not only provide a resource for developing microbial materials that enhance atmospheric CO₂ fixation and for applications in agriculture, the environment, and artificial photosynthesis, but will also be an important research material for deepening our understanding of the evolution of photosynthesis.

The aim of this research is to develop a method for efficiently screening photosynthetic bacteria utilizing microdroplets, using near-infrared autofluorescence at 800–900 nm emitted by photosynthetic bacteria that contain bacteriochlorophyll as an indicator (this technology is currently under development).



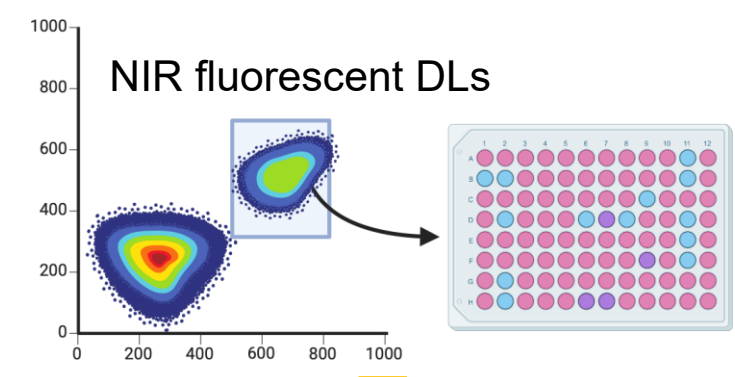
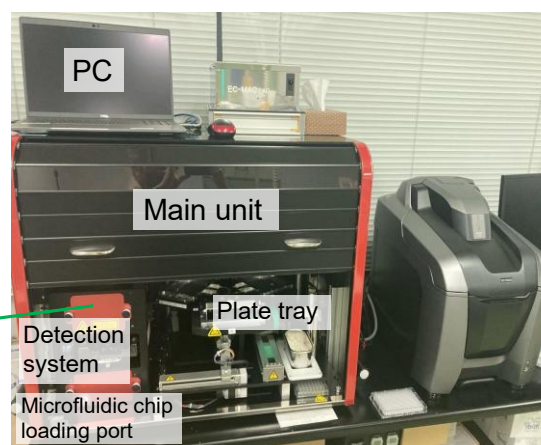
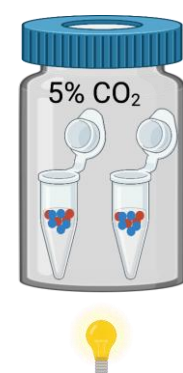
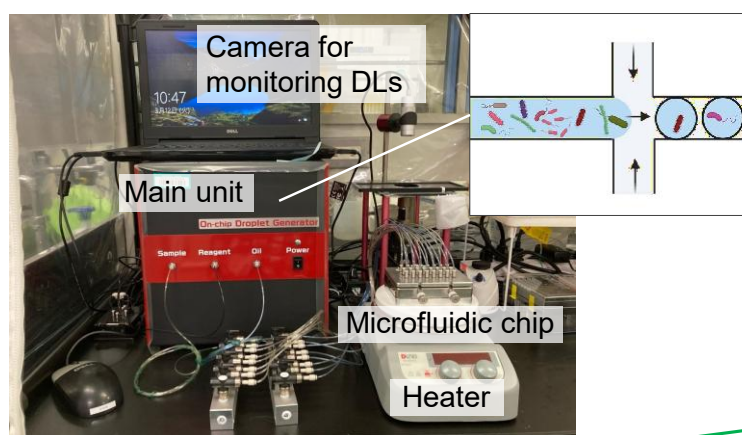
Screening Strategies

Encapsulation of soil microbes
Production of microdroplets (DLs)
(Droplet Generator)

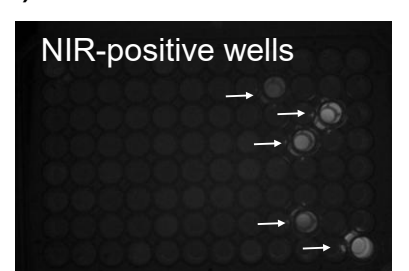
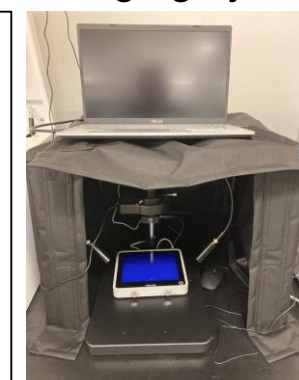
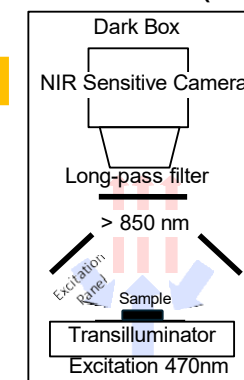
DL-based culture
(Light/CO₂)

1st screening
Sorting and dispensing of
DLs containing proliferated
photosynthetic bacteria
(Droplet Selector)

Culture using a microplate
with DLs dispensed
(Light/CO₂)



2nd screening
Detection of wells containing
proliferated photosynthetic bacteria
(NIR imaging system)



Verification using photosynthetic bacteria *Rhodopseudomonas palustris* and *E. coli*

<Methods>

Gel preparation

- Dissolved 3% low-melting-point agarose gel (2 ×) in water.

Preparation of culture medium

- Pre-culture
- Adjusted the concentration to 80 cells/DL.

Mixing the gel and culture medium

- Mixed equal amounts of the gel and culture medium.
- Filtered it through a 5-μm filter.

Production of DLs with the Droplet Generator

- Used the 2D Chip-1100DG.

DL-based culture

- Cultured in the W/O DLs (irradiated with light).

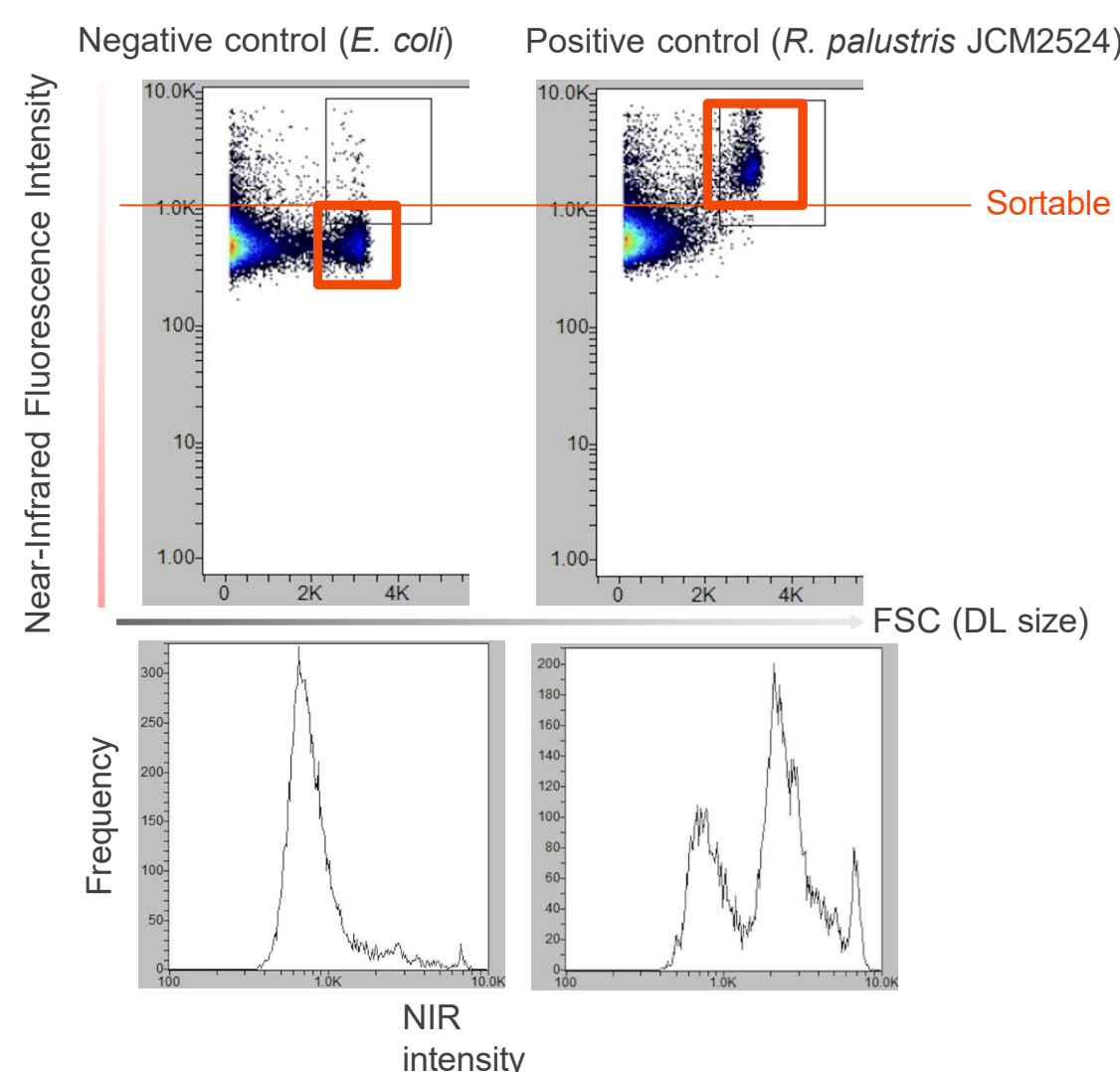
Breaking up the DLs

- Added 10% PFO, the weight of which was equal to that of the DLs, and mixed them upside down.
- Added a medium with a weight equal to that of the DLs.
- Broke up the DLs with a plasma ball.
- Collected the DL layer.

Sorting and dispensing with the Droplet Selector

- Verified whether sorting was possible with the NIR detection system.
- Dispensed the DLs into a microplate and cultured.

<Results and challenges>



The DLs containing the photosynthetic bacteria as a positive control and *E. coli* as a negative control were detectable at levels that allowed sorting with the introduced GaAs-based near-infrared detection system.

However, after they were dispensed in a microplate and then cultured, no growth was observed. It was thought that the cells encapsulated in the gel droplets may not have migrated into the culture medium, or that they may have been killed by PFO when the droplets were broken.