

Isolation of Candidate Antagonistic Bacterial Strains against *Vibrio harveyi* Pathogenic to Kuruma Shrimps by High-throughput Screening Using a Microdroplet Production Technique

Hajime Yuasa¹, Yuri Ota²,

Kayo Konishi¹, Miho Furukawa¹, Reiko Nozaki¹, Hidehiro Kondo¹, Ikuo Hirono¹, and Keiichiro Koiwai¹

¹ Tokyo University of Marine Science and Technology, ² On-chip Biotechnologies Co., Ltd.

Background

Bacterial infections caused by pathogenic bacteria such as *Vibrio* spp. have been a problem in aquaculture of kuruma shrimps. As a potential solution, **biological control** using antagonistic bacteria that can inhibit the growth of pathogenic bacteria and kill them has attracted attention. It has been reported that bacteria such as *Bacillus* spp. and lactic acid bacteria exhibit antagonistic properties against pathogenic bacteria in fisheries. On the other hand, the inefficiency of the conventional screening method for antagonistic bacteria, streaking-based co-cultures on agar plate media, has been a bottleneck in the practical application of this biological control method.

Recently, a high-throughput screening method for antagonistic bacteria has been developed, utilizing a technique that generates microdroplets ranging from 30 to 100 μm in diameter. This method can generate approximately 100,000 droplets per minute and is expected to be highly efficient compared to conventional screening methods. However, this screening method has been applied to a limited number of pathogens, and there are no reports of its application to pathogenic bacteria that are problematic in aquaculture target organisms.

The objective of this study was to develop a microdroplet-based screening method for antagonistic bacteria against pathogenic bacteria for kuruma shrimps. *Vibrio harveyi*, one of the pathogenic bacteria for kuruma shrimps, was used as a model organism.

1. Preparation of fluorescent protein-expressing *V. harveyi*

Transformation based on homologous recombination

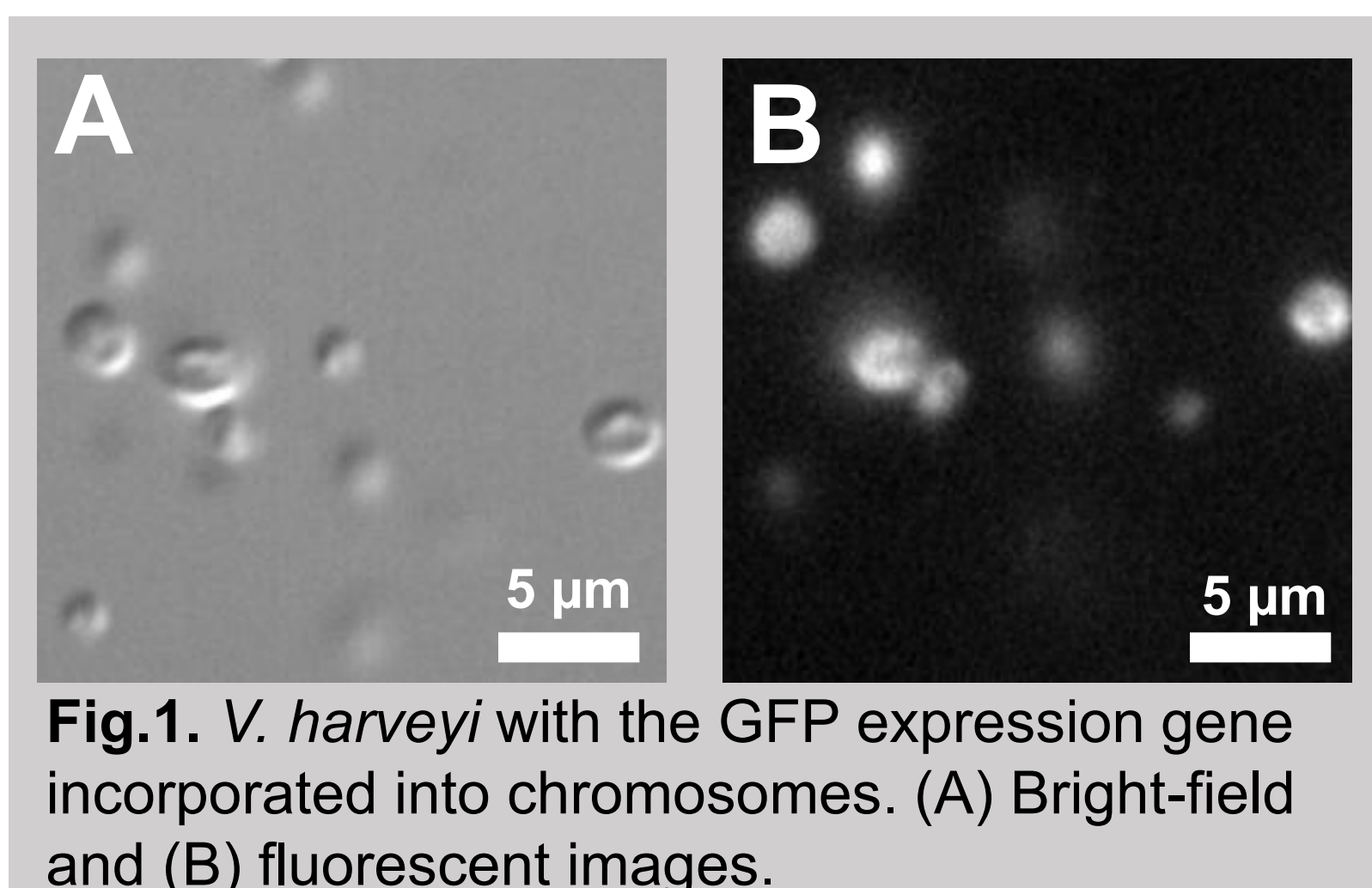
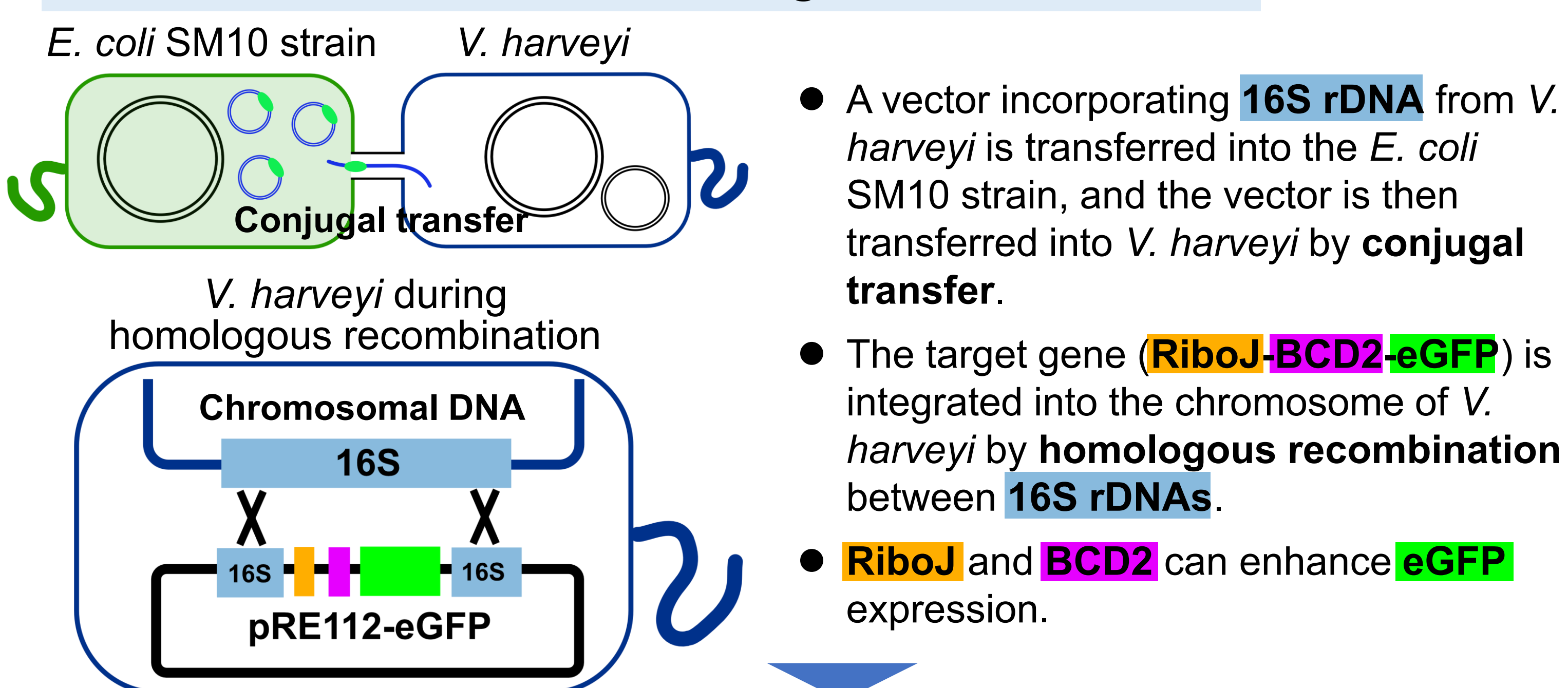


Fig.1. *V. harveyi* with the GFP expression gene incorporated into chromosomes. (A) Bright-field and (B) fluorescent images.

✓ The GFP-expressing strain was successfully prepared, but the growth rate was slower than that of the wild-type strain. ➡ We will prepare fluorescent strains with little phenotypic difference from wild-type strains.

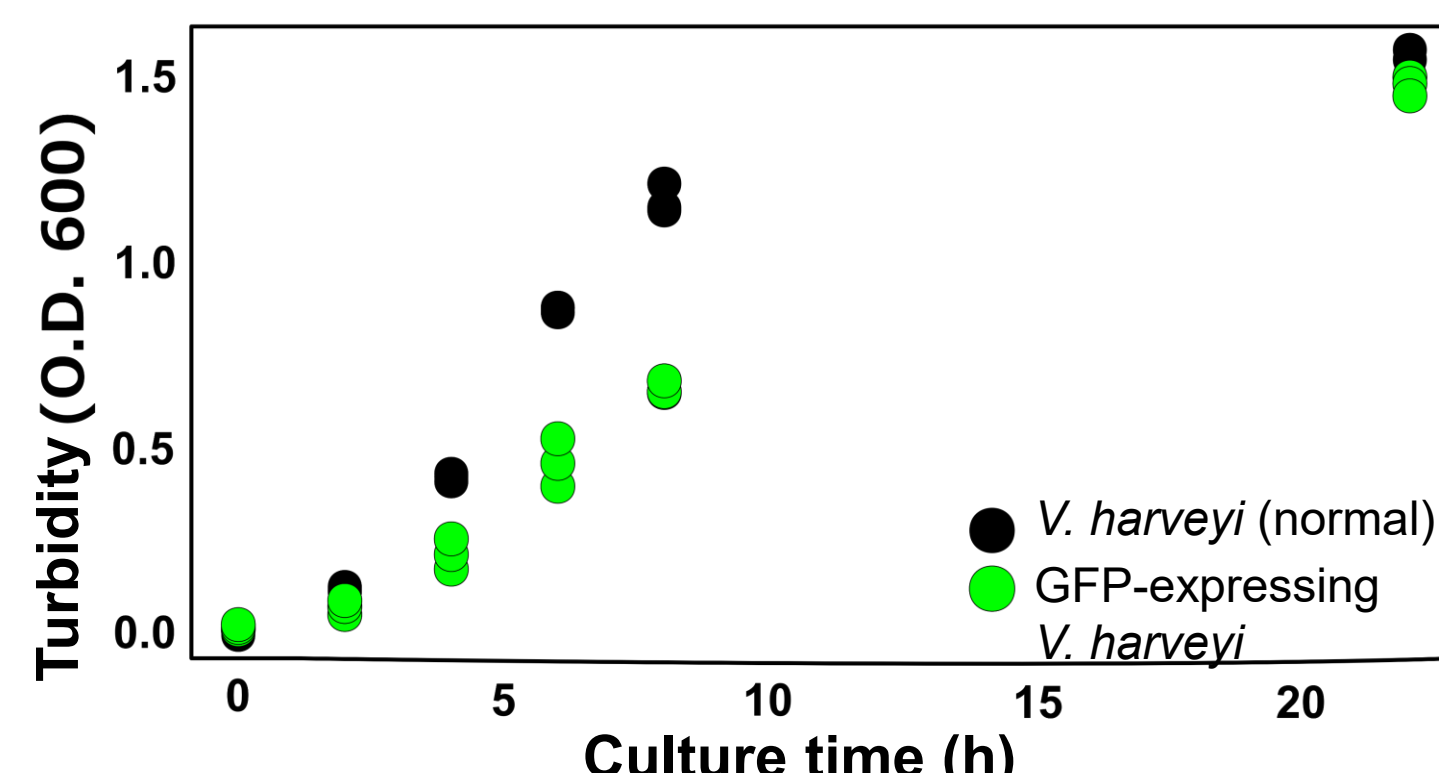
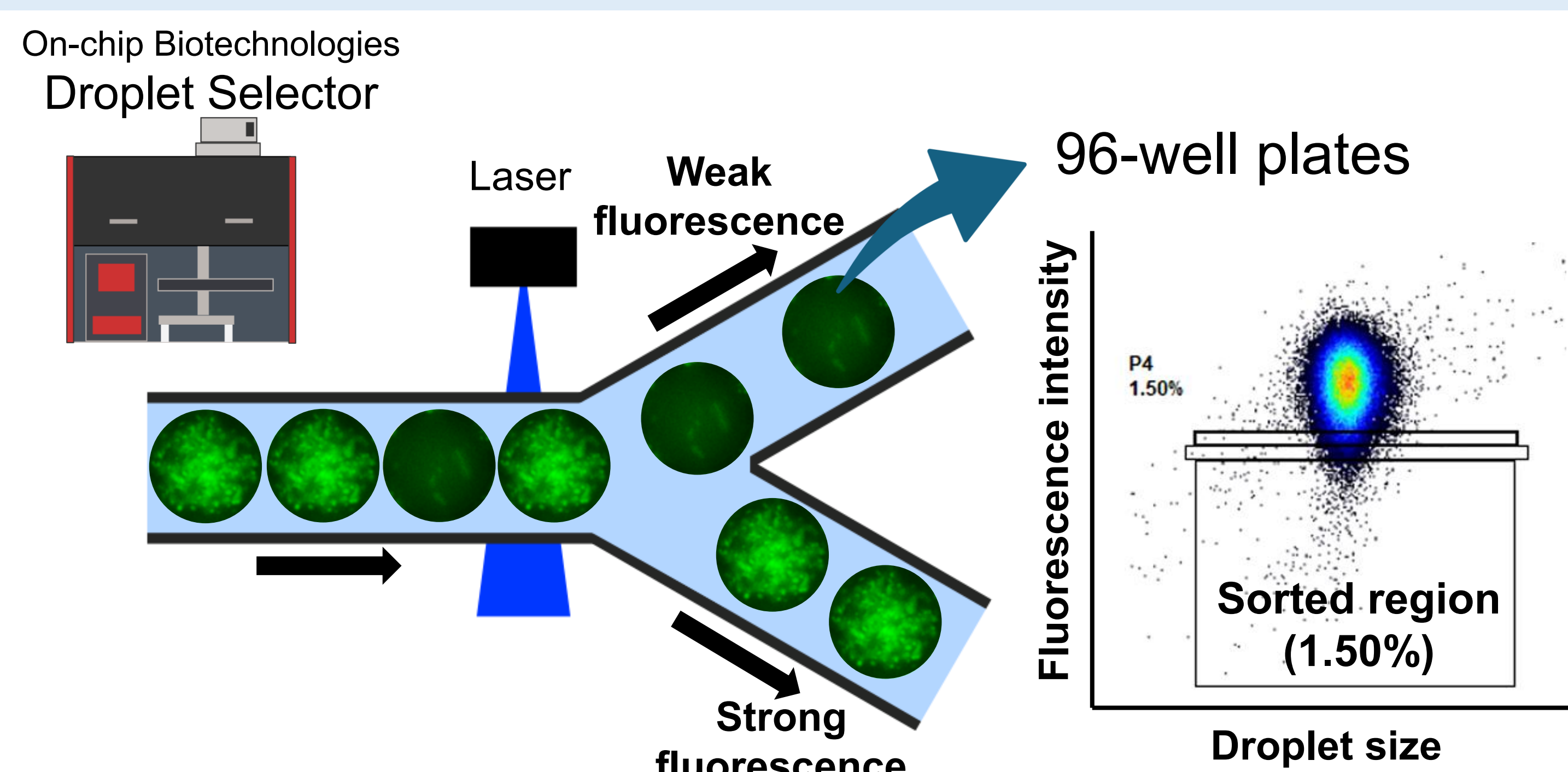


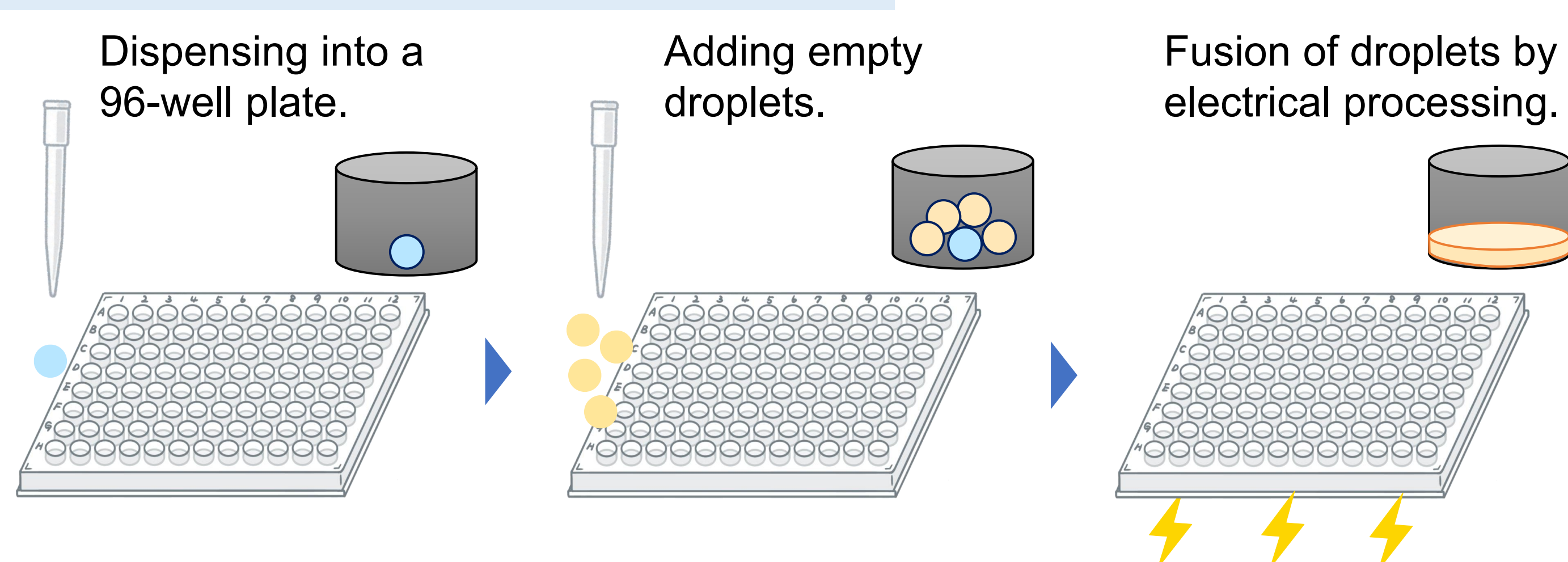
Fig. 2. Comparison of growth rates of normal and fluorescent strains based on spectrophotometry.

3. Separation of quenched droplets

(1) Separation of quenched droplets based on fluorescence intensity



(2) Re-culture of bacteria in droplets



2. Co-culture in microdroplets

(1) Co-encapsulation

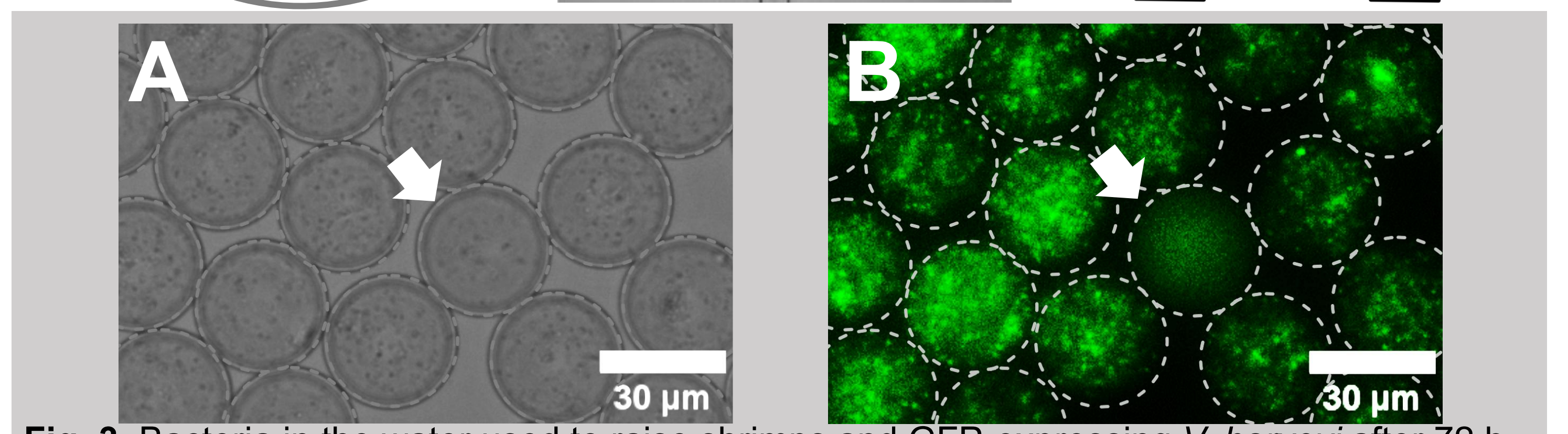
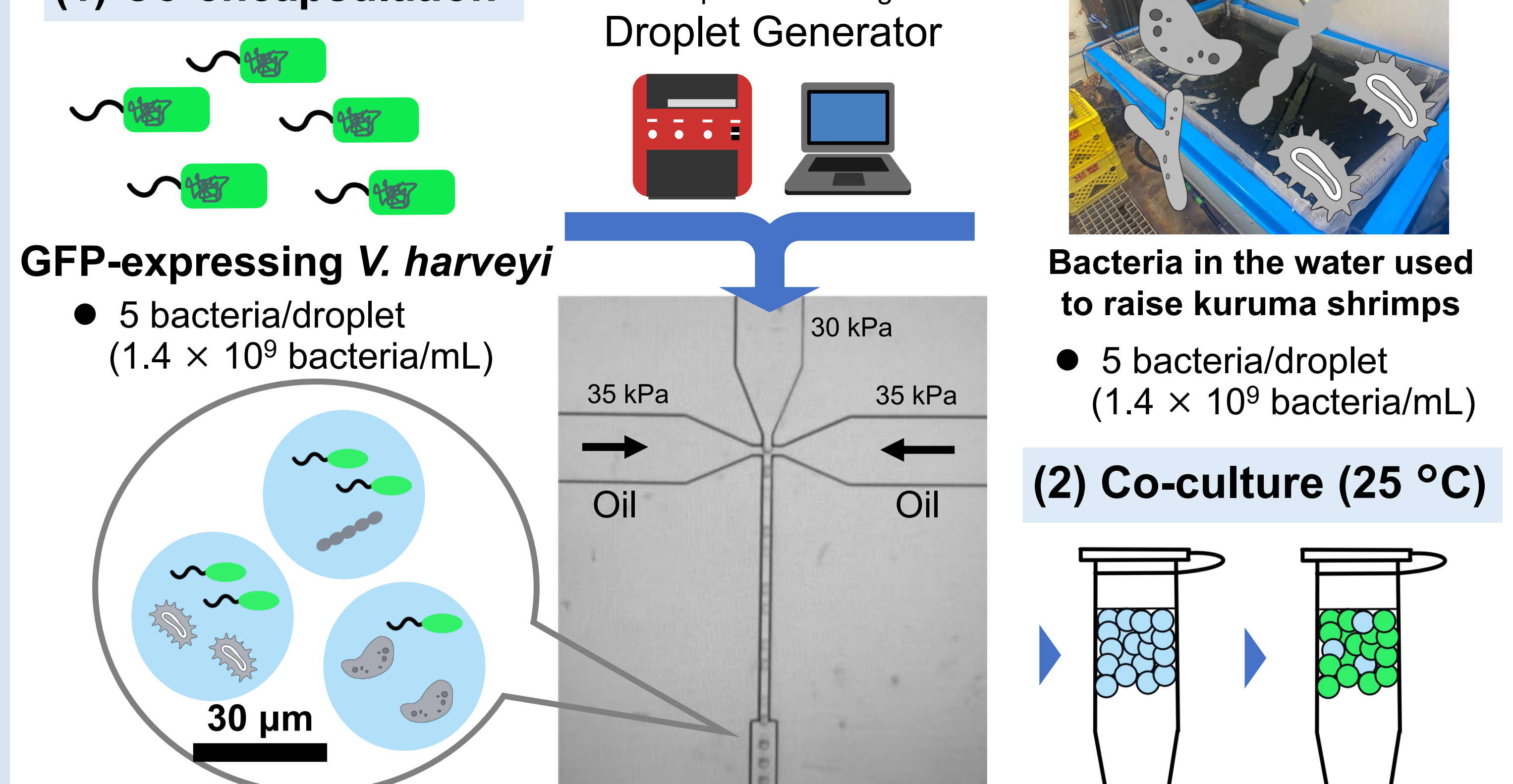


Fig. 3. Bacteria in the water used to raise shrimps and GFP-expressing *V. harveyi* after 72 h of co-culture. (A) Bright-field and (B) fluorescent images.

✓ We confirmed that there were droplets with relatively low fluorescence intensity by co-culturing with bacteria in the water used to raise kuruma shrimps.

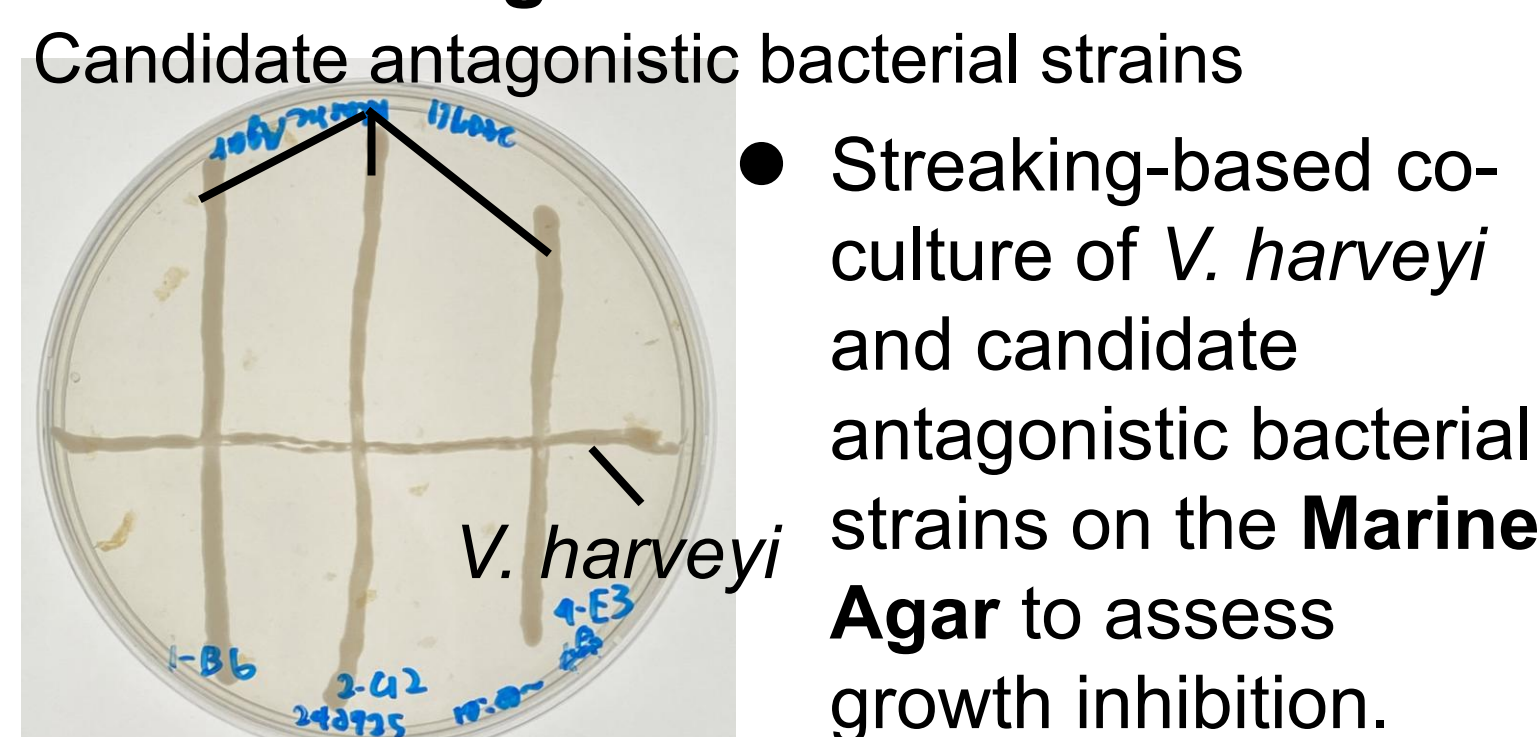
4. Analysis of candidate strains of antagonistic bacteria

(1) 16S rDNA gene sequencing

- We obtained candidate strains of antagonistic bacteria (3 genera, 24 strains).

(2) Re-evaluation of antagonistic properties

Streaking-based co-culture



Co-culture in droplets

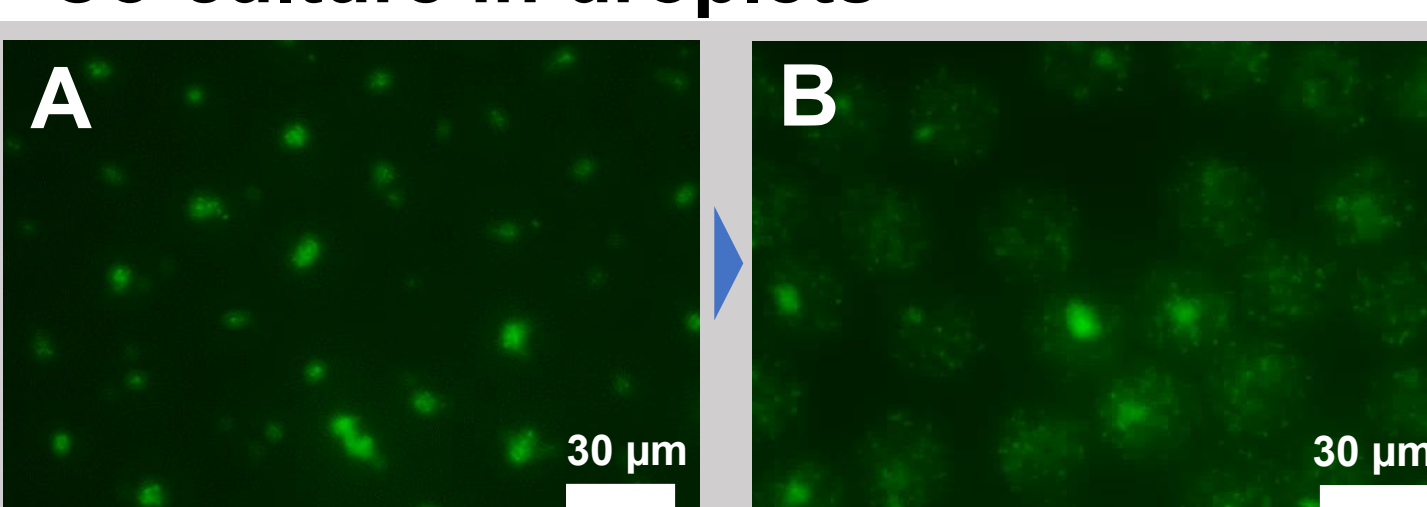


Fig. 4. Culture of GFP-expressing *V. harveyi* in droplets. (A) 0 h, (B) 22 h.

Co-culture in liquid media

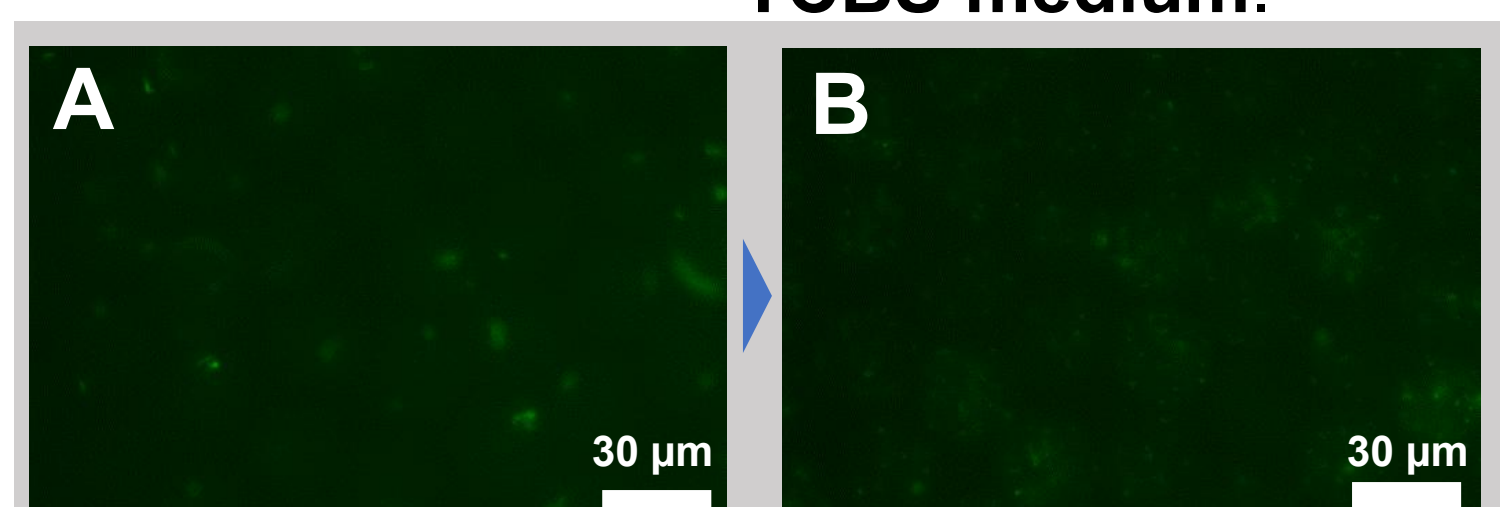
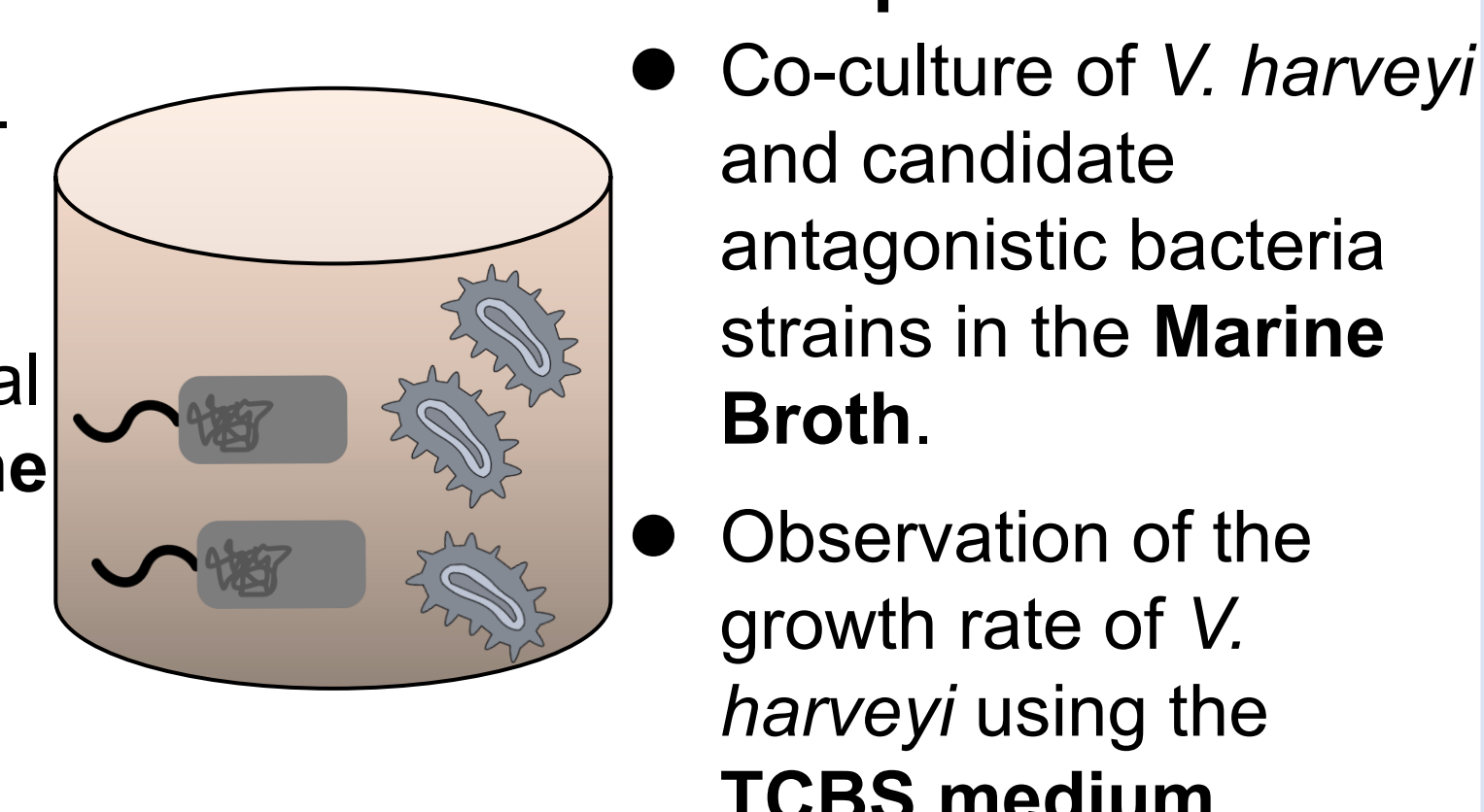


Fig. 5. Co-culture of GFP-expressing *V. harveyi* and candidate antagonistic bacterial strains in droplets. (A) 0 h, (B) 22 h.

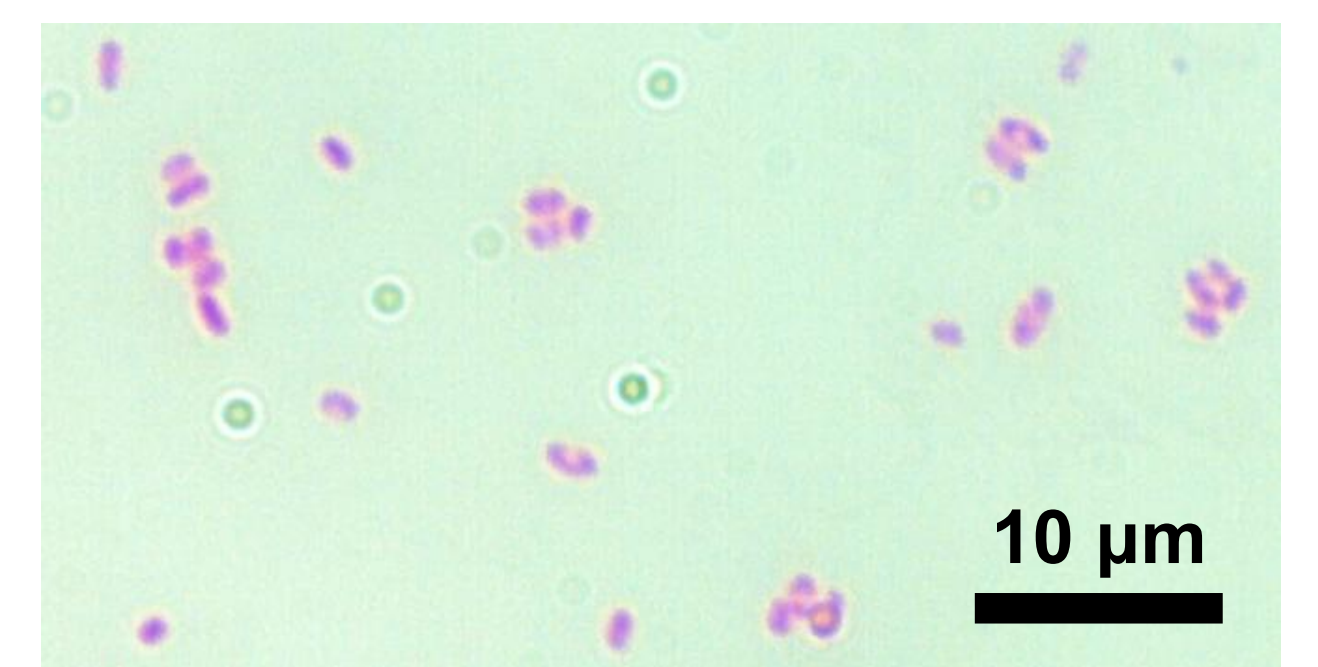
✓ We successfully isolated candidate strains of antagonistic bacteria (3 genera, 24 strains) and confirmed the growth inhibition effect of some of these strains on *V. harveyi* in droplets; however, antagonism was not observed when agar or liquid media were used.

➡ We will re-examine the screening and test-retest methods for antagonism and attempt to obtain new candidate strains of antagonistic bacteria.

Acknowledgements

We would like to express our sincere gratitude to On-Chip Biotechnologies Co., Ltd., Associate Professor Masahito Ishikawa of the Environmental Synthetic Biology Lab at Nagahama Institute of Bio-Science and Technology, Yasunori Ichihashi (Team Leader), and Megumi Narukawa (Development Researcher) of the Plant-Microbe Symbiosis Research and Development Team of RIKEN for their valuable guidance and advice on this research.

Vibrio harveyi TUMSAT-2019



- Isolated at a kuruma shrimp farm on Kume Island, Okinawa in 2019 and sequenced for the entire genome (Yuasa, H. *et al.*, 2024).
- A pathogenic bacterium that causes vibriosis and acute hepatopancreatic necrosis in kuruma shrimps.