

Optimization of Filamentous Fungi for Culture Using GMDs

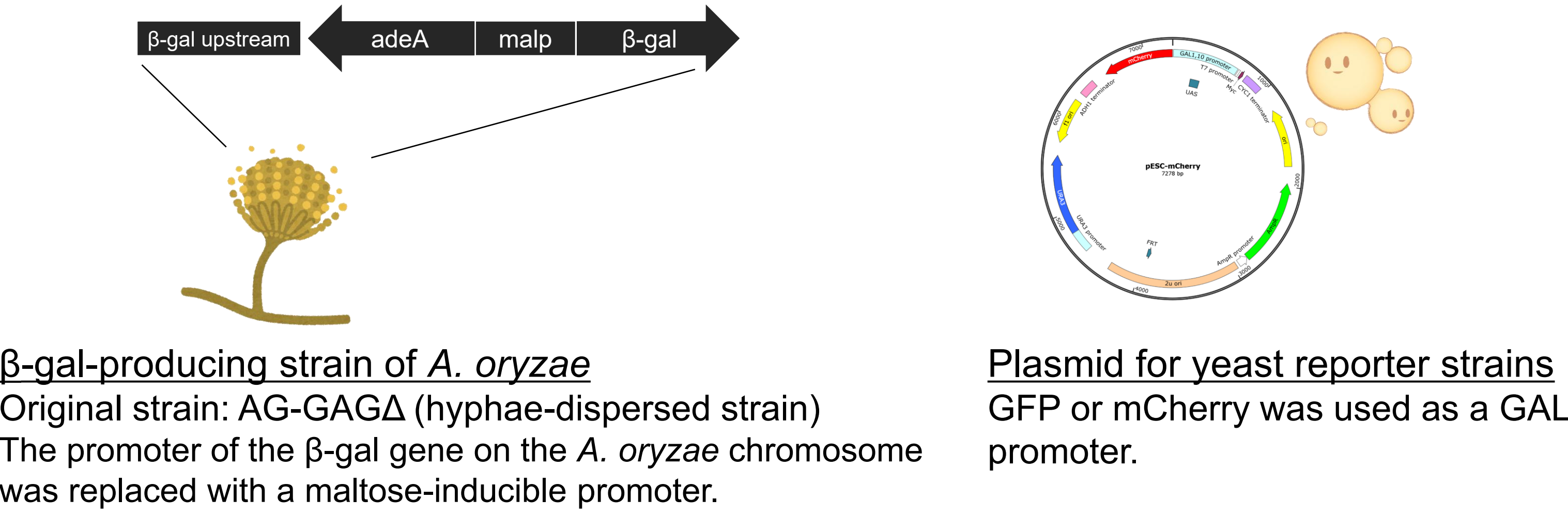
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Abstract

It is estimated that ≥3 million species of filamentous fungi exist on Earth. Some of them are industrially utilized due to their capacity for high production of enzymes and low molecular weight compounds, and there is a great need for mutation breeding of them. Gel microdrop (GMD) technology has been adopted for large-scale screening of mutant strains of microorganisms. However, filamentous fungi, which are multicellular microorganisms that grow in a filamentous form with cells connected in series, are difficult to be cultured in GMDs because their hyphae adhere to each other to form pellets. In this study, we focused on *Aspergillus oryzae*, an industrially important filamentous fungus, and used a mutant strain with well-dispersed hyphae to investigate the optimal conditions for conidia preparation, selection, and cultivation using GMDs. To investigate the culture conditions, we attempted to standardize the lag time required for germination and uniformity of hyphal extension. We also attempted to construct a system for obtaining an *A. oryzae* strain producing high levels of β-galactosidase (β-gal) by using GMDs and a yeast reporter strain, in order to screen for a mutant strain producing enzymes at a high level.

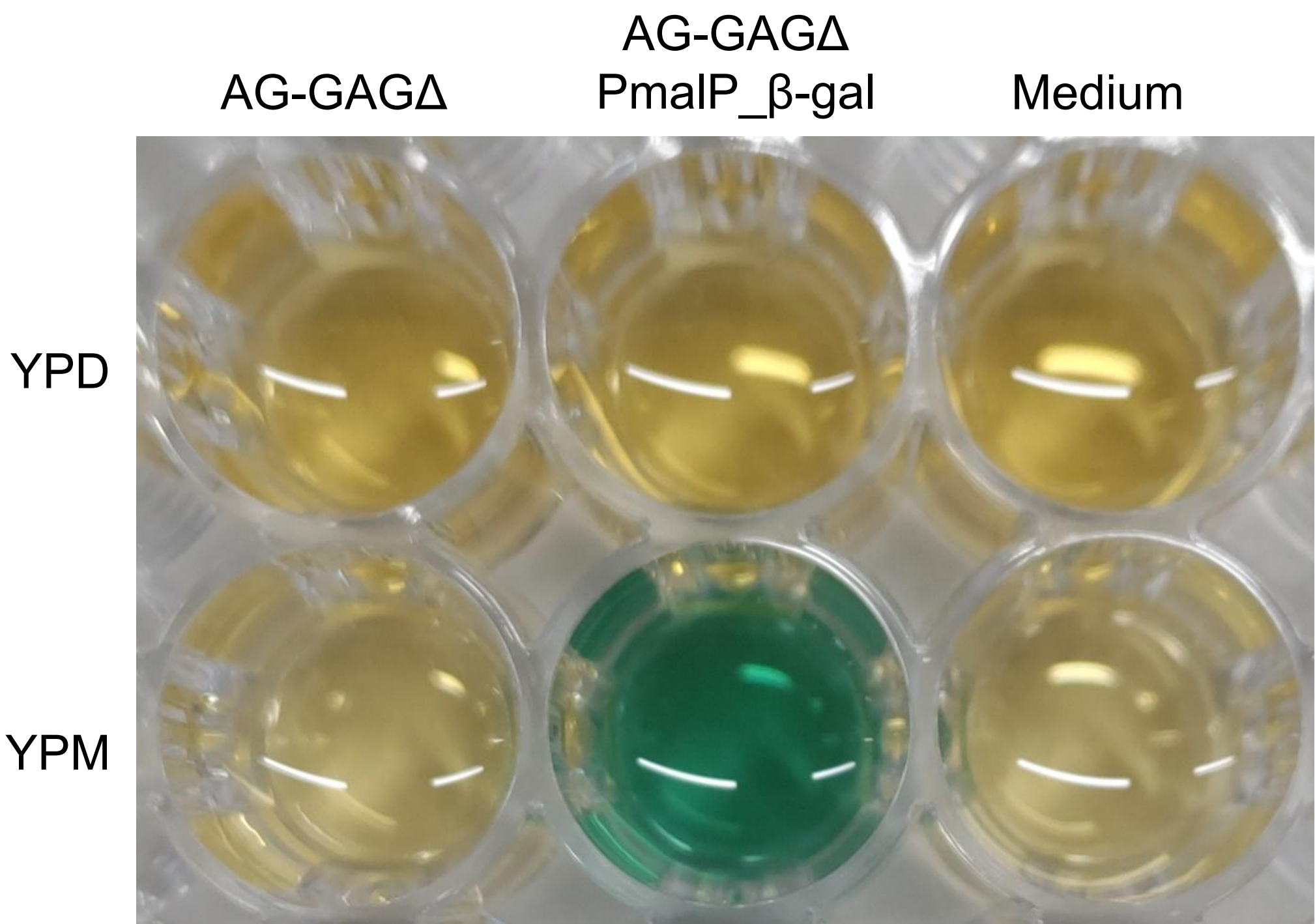
Detection of β-galactosidase (β-gal) production by *A. oryzae* using a reporter yeast



Co-culture

The maltose in the medium induces *A. oryzae* to produce β-gal, which breaks down the lactose in the medium into galactose and glucose, causing the yeast reporter strain to emit light from GFP or mCherry.

Confirmation of β-gal in the culture supernatant



Spores were inoculated into 50 mL of liquid medium.

↓
The culture was incubated at 30 °C with lateral shaking for 24 hours.

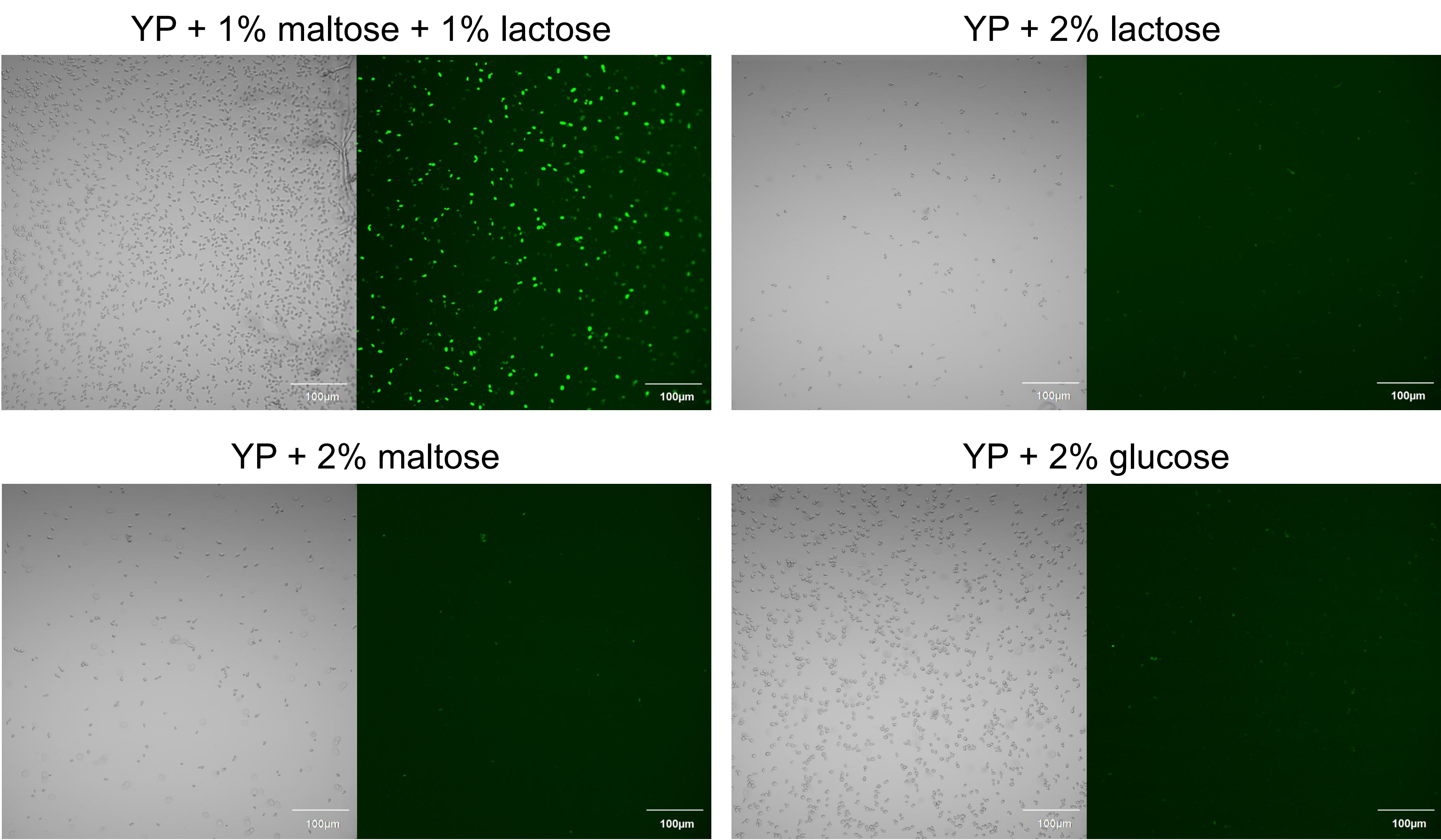
↓
The culture medium was collected.

↓
5 μL of X-gal (40 mg/mL) was added to 1 mL of the medium.

↓
It was kept in the dark at room temperature for 1.5 h.

β-gal production was induced specifically in the culture using maltose.

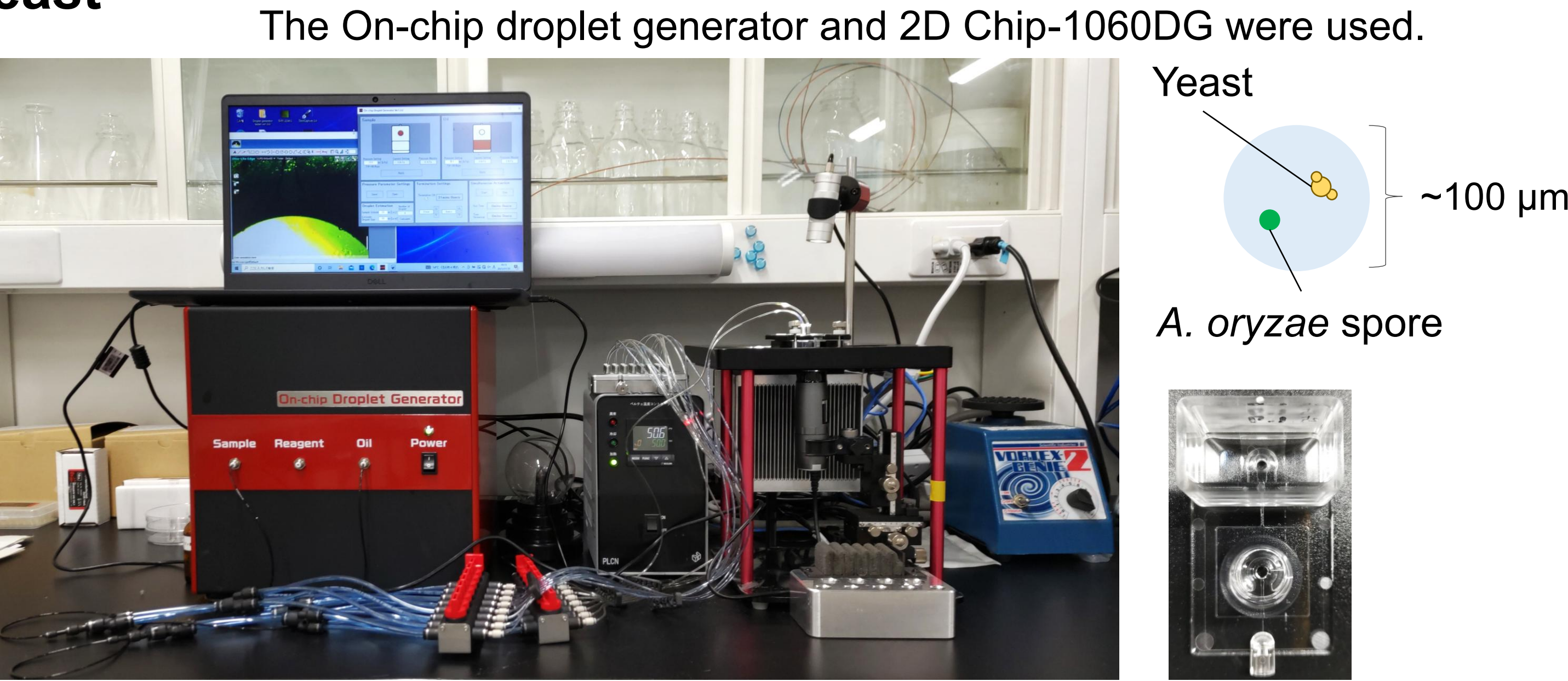
Co-culture of the AG-GAGA Pmal-βgal strain and the reporter yeast



We inoculated the strains into each medium and incubated at 30 °C with shaking overnight.

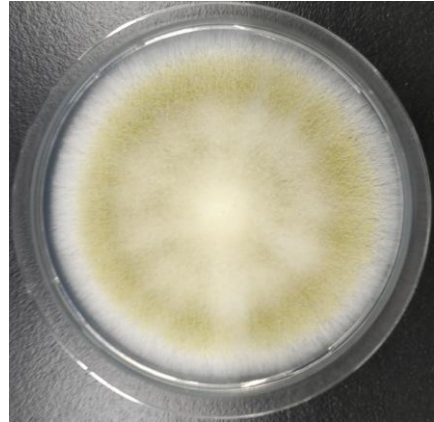
β-gal, whose production was induced in *A. oryzae* by the presence of maltose in the medium, degrades lactose in the medium to produce galactose, which in turn induces the expression of GFP in the yeast with the GAL1 promoter.

Preparation of GMD containing *A. oryzae* spores and reporter yeast



For *A. oryzae*
Induced sporulation by plate culture.
↓
Prepared a spore suspension and counted the number of spores.

For yeast
Incubated overnight in an SD-ura liquid medium.
↓
Counted the number of cells.



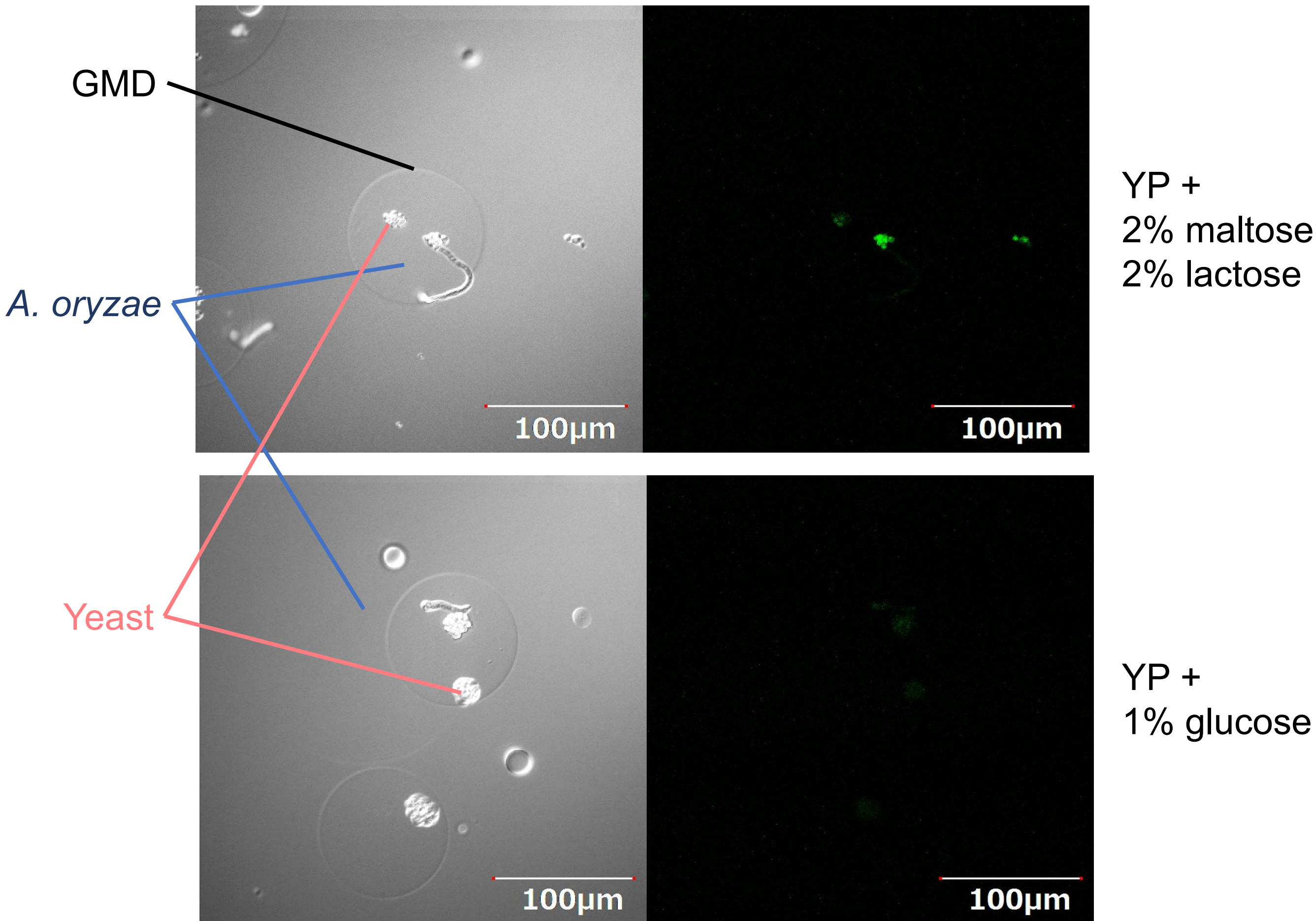
A. oryzae: 5×10^6
Yeast: 1×10^7
Added them to a single tube and suspended in 1 mL of liquid medium.

Mixed a 2% agarose gel solution (agarose dissolved in a culture medium) with the cell suspension in a 1:1 ratio to prepare GMDs.

Solidified the agarose on ice and then cultured the mixture by shaking at 30 °C in an emulsion state.

Collected the GMDs, observed them under a microscope, and sorted them using a cell sorter.

Co-culture of the β-gal-producing strain of *A. oryzae* and the yeast reporter strain



Prepared GMDs by mixing the β-gal-producing *A. oryzae* strain with the yeast reporter.

↓
Incubated in emulsion at 30 °C with shaking for 8 hours.

↓
Broke the emulsion after incubation, removed the GMDs, and observed them under a microscope.

Summary

We constructed a β-gal-producing *A. oryzae* strain and confirmed β-gal production.

Co-culture of the *A. oryzae* strain with a yeast reporter strain was performed and it was confirmed that the reporter strain responded to the production of β-gal.

The *A. oryzae* strain and the reporter yeast were co-encapsulated in GMDs and co-cultured.

We confirmed that the reporter yeast emitted light even within the GMDs in response to β-gal production by *A. oryzae*.

Future works

We will check if the GMDs containing two strains can be detected by the On-chip sorter.

Sorting will be performed to determine if the desired GMDs can be recovered.

Mutagenesis of *A. oryzae* by UV or other means will be performed to obtain strains with enhanced β-gal production.