Training report on JICA's KCCP The project for Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy (Be-HoBiD)

At Division of Biosphere Sciences, Graduate School of Environmental Sciences, Hokkaido University

Ms. Juthaporn Phonmakham

Kasetsart University

24 October – 25 November 2022

Acknowledgement

First, I would like to thank scholarship from JICA for support and Professor Masaaki Morikawa and his lab members for take care and help while I was there.

From 24 October to 25 November 2022, I trained at Hokkaido University.I learned a lot about how to Duckweed – microbe interaction, Amino acids analysis and vertical farming, I wrote my report as follows.

24-28 October 2022

Duckweed – microbe interaction

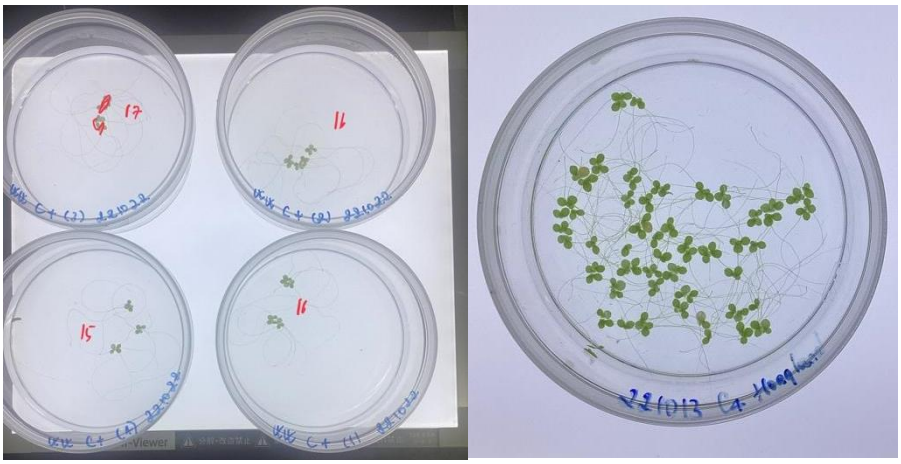
- Hoagland, MA liquid and R2A agar preparation

I learned how to Hoagland, MA media and R2A agar preparation.

For Hoagland media will be used to culture duckweed, MA media used to culture microalgae and R2A agar for culture bacteria.

-How to learn about counting the number of fronds of duckweed after co-cultivated with bacteria and how to measure dry weight

For co-cultivation of duckweed and bacteria with microalgae, the number of fronds was counted and take a photo every 2 days and the dry weight was determined at 14 days. The dry weight of duckweed was incubated at 60 °C for 2 days.



Count the number of fronds and take a photo

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31 October – 4 November 2022

Duckweed – microbe interaction

-How to learn the method for co-cultivation duckweed and bacteria with microalgae

I learned to co-culture duckweed and bacteria. The first step is bacterial culture. We cultivated bacteria into R2A medium for 24 hours and then measured OD600 to determine the concentration of bacterial cells. After that, the bacteria cells were diluted OD600=0.3 and then put the duckweed into cell suspension and incubated at 28 ºC for 24 hours.

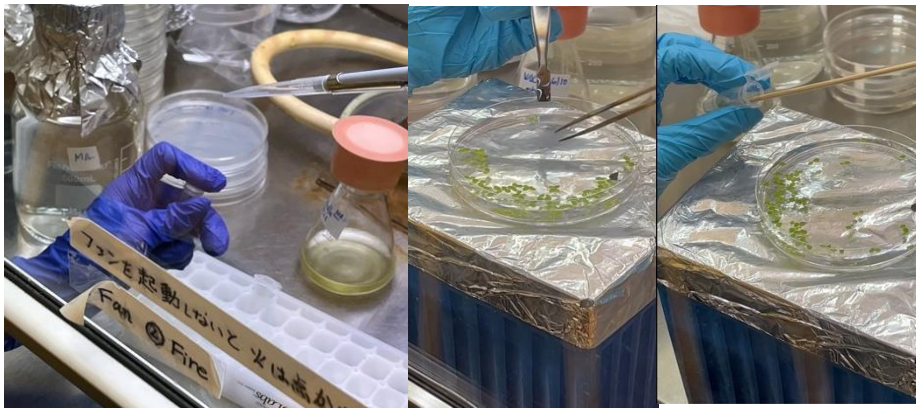
Preparation MA and WW medium with microalgae

The concentration of microalgae was measured at OD 680 nm and diluted at OD680=0.0001 in MA and WW medium.

Wash with sterile distilled water, separation fronds of duckweed

Because the duckweed has daughters' fronds If it is attached, it will affect the experiment. After that, take ten fronds and take them to the CFU count. And take 2 fronds into 50 ml MA or WW medium with microalgae at OD680 nm = 0.0001. Incubated at 28 ºC and count the number of fronds and take a photo every 2 days and the dry weight was determined at until 14 days.

The above step is different from my experiment. I can apply that to my future experiment.



7-11November 2022

-Amino Acid Analysis

Staff demonstrates the process of sample preparation before amino acid analysis with an amino acid analyzer and learns to working principle of an amino acid analyzer.

For learning Amino acid analysis are new knowledge. This knowledge can be adapted and useful for my future experiments.

-**Surface sterile** (Duckweed)

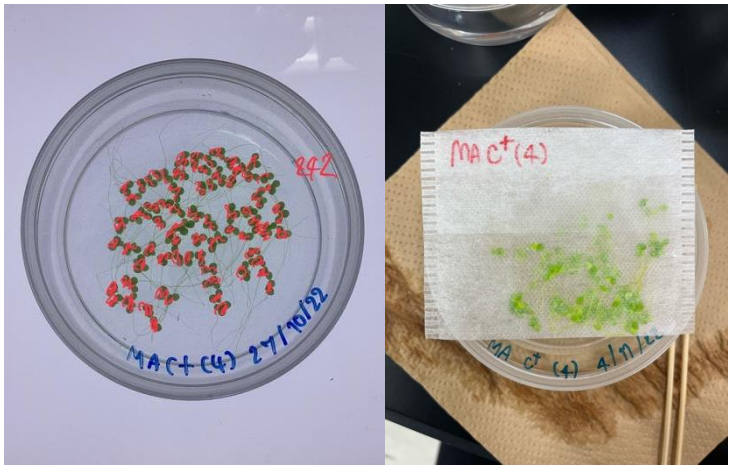
In this experiment, we used 5% bleach to sterile duckweed. Place about 10 fronds of duckweed into a 1.5 ml tube containing 5% bleach and then shake for 2 minutes and wash with sterile water 2 minutes 2 times. After that, pick 1 frond of duckweed transfers to a 12 well plate containing 3 ml of NF medium+1% sucrose. Incubated at 28 ºC for 1-2 days. If there is no contaminate NF medium is clear, if contaminated NF medium is turbid. Transfer sterile duckweed into NF medium without 1% sucrose. I can apply to my future experiment.

14-18 November 2022

Duckweed – microbe interaction

PGPB is a continuation of last week's experiment. Counting the number of fronds, take a

Pictures and measures dry weight



Count the number of fronds and measures dry weight

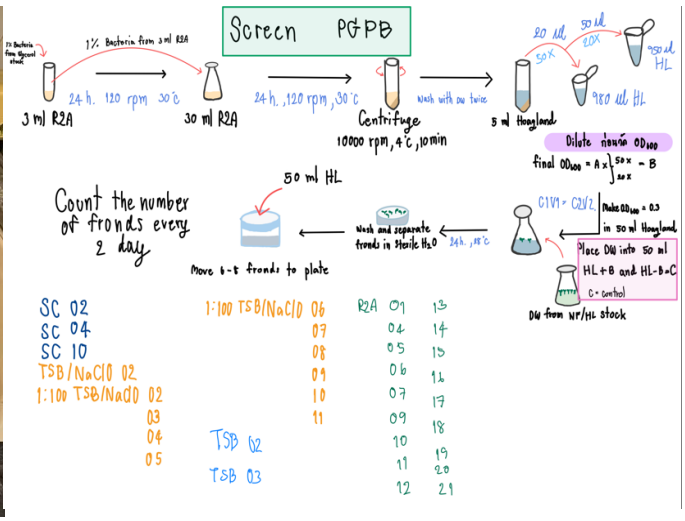
- **Amino acid analysis**

For amino acid analysis, Staff perform demonstrations from real samples. The samples were digested with hydrochloric acid. After that melting glass containers using high-temperature fire, and use liquid nitrogen to freeze the samples. Remove bubbles with a cool blower and close the glass by using high heat, hydrolysis by incubation at 110 °C for 24 h. The reaction was stopped at -30 °C for 10 min and then centrifuge evaporation at 60 °C for 40 min. The sample was filtered with a 0.22 μm filter. 40 μl of the sample was injected into the amino acid analyzer.

21-25 November 2022

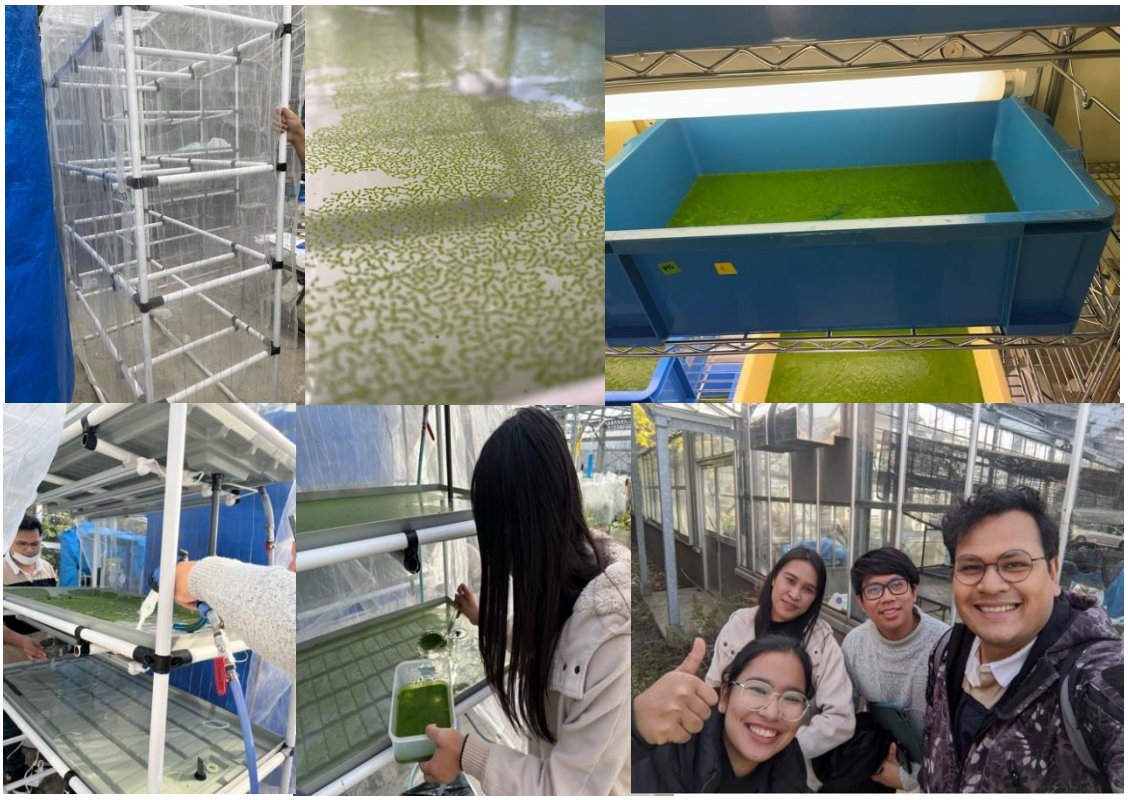
- Screening of plant growth promoting bacteria (PGPB)

The purpose of screening PGPB is to identify bacteria which may promote the growth of *Lemna aequinoctialis* First step we cultured 1% bacteria from glycerol stock into test tube with 3 ml R2A liquid and then incubated with shaking incubator 120 rpm at 30 ºC for 24 h. After that take 1% bacteria from 3 ml R2A into 30 ml R2A liquid and then incubate with a shaking incubator 120 rpm at 30 ºC for 24 h. For harvesting cells, we transfer to a conical tube and centrifuge at 10000 rpm 4 ºC for 10 minutes and wash with sterile water 2 times. For the optical density (OD) at 600 nm, we adjust OD600=0.3 with Hoagland medium, after that place duckweed into 50 ml Hoagland medium with bacteria cell OD600=0.3 incubated 28 ºC for 24 h. And then wash and separate fronds in sterile water and transfer 6-8 fronds of duckweed into 50 ml Hoagland medium without bacteria, incubated at 28 ºC for 14 days and count the number of fronds every 2 days, for the dry weight was determined at until 14 days. I wrote a summary of this experimental process as shown in the figure. For this experiment, I can apply that to my future experiment.



- Vertical farming

I have learned to growing *wolffia* in ideal conditions on a large scale. In addition, we learned the harvesting of *wolffia* and measuring growth rates with ImageJ program because *wolffia* are so small they are so hard to count.



For this training, I learned a lot topics, some ideas, and skills on co-culture and surface sterile, Amino acid analysis, vertical farming and how to measure the growth rate of duckweed. I can use all of this knowledge on my experiments.

In addition, for the weekend I have to traveled to many places in Hokkaido. Autumn season turning to winter season it very exciting for me, changing color of leaves it so beautiful. I training at Hokkaido for 1 months make me good memories. I would like to thank scholarship from JICA for support and I would like to thank Professor Masaaki Morikawa and his lab members for Take care and help while I was there