Report for training 2022

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Introduction

This training is under the joint research between Japan and Thai counterparts for 5 years (2021-2026) under a multi-disciplinary project entitled “Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy” implemented under the “Science and Technology Research Partnership for Sustainable Development (SATREPS)”. This collaboration is supported by Japan International Cooperation Agency (JICA) in collaboration with Japan Science and Technology Agency (JST). I, Peerapat Roongsattham, had been trained under course title “Duckweed Holobion interaction and function enhancement” from 5 November 2022 to 26 November 2022 at Osaka University in Professor Ike laboratory and at Kyoto University in Professor Oyama laboratory.

Objectives

1. To learn a series of basic techniques and knowledge necessary for studying about the duckweed-microbe interaction.
2. To learn a series of basic techniques and knowledge necessary for the handling and preservation of duckweed.

Expected outputs

1. The trainee can cultivate a variety of duckweed and analyze the bacterial community in their rhizophere. The trainee can evaluate the effects of augmented bacteria on duckweeds concerning biomass production and/or water purification capability.
2. The trainee will be able to handle and preserve a variety of duckweeds in the laboratory, and analyze gene function in duckweed.

Expected outcomes

1. The trainee will gain experimental techniques and knowledge that should promote the research and technology development for enhancing the function of duckweed holobionts in Thailand. Publication of a research paper in international collaboration is also expected.
2. The trainee will apply the technique obtained in Japan to establish a duckweed (-holobiont) collection center in Thailand.

Training in Japan

In Osaka University

On the first day (7 November), Prof. Ike introduced his laboratory and Osaka University.

On the second day (8 November), We started with A&H medium preparation in the morning with the recipe in Table 1. Then, in the afternoon, we collected environmental water sample from a pond in the University and collected microorganism from the water sample and also did duckweed-microorganism co-cultivation.

Table 1: Arnon & Hoagland modified recipe

Arnon & Hoagland modified				
ingredient	molecular weight	Final concentration(mg/L)	Final concentration(mol/L)	stock number
KNO_3	101.1	36.1	0.000357072	1
NaH_2PO_4	120	3.87	0.00003225	7
K_2SO_4	174.3	293	0.00168101	2
$MgSO_4 \cdot 7H_2O$	246.4	103	0.000418019	3
$CaCl_2 \cdot 2H_2O$	147.1	147	0.00099932	4
$FeSO_4 \cdot 7H_2O$	277.9	3.33	1.19827E-05	5
H_3BO_3	61.8	0.95	1.53722E-05	6
$MnCl_2 \cdot 4H_2O$	197.9	0.39	1.97069E-06	6
$CuSO_4 \cdot 5H_2O$	249.6	0.03	1.20192E-07	6
$ZnSO_4 \cdot 7H_2O$	287.5	0.08	2.78261E-07	6
$Na_2MoO_4 \cdot 2H_2O$	241.95	0.34	1.40525E-06	6
EDTA・2Na	372.24	5	1.34322E-05	5.5
KOH				

On 9 November, Lab members in the lab and I gave a talk about our research in the morning and in the afternoon, I prepared R2A medium.

On 10 November, Prof. Ike, Inoue and Ishizawa had a talk about our lab and possible collaboration. After that, Prof. Ishizawa took me and Ms.Yuparat to Himeji castle and to Hyogo University to visit his laboratory.

On 11 November, we did microorganism recovery from duckweed and plating to R2A medium. We also did DNA extraction for amplicon sequencing. Finally, we also used data prepared by Ms.Sugiyama for 16S rRNA sequencing data analysis as shown in Fig. 1

X	Y	Z	AA	AB	AC
	SampleA	SampleB	SampleC	SampleD	SampleE
Comamonadac	0.183900378	0.150898204	0.557018523	0.372310073	0.716080928
ACK-M1	0.373082055	0	0.017881053	0	0.001926863
Methylophilac	0.076300867	0.250299401	0.000892658	0.06724213	0
Rhodocyclacea	0.001751167	0.009580838	0.036766347	0.041056144	0.141812927
Sphingomonad	0.005058928	0.010778443	0.089126311	0.059093767	0.034243706
Caulobacterac	0	0.086227545	0.006750725	0.005996421	0.001089096
Oxalobacterac	0.070241272	0	0.053280518	0.068910489	0.029112386
C111	0.044335112	0	0.003849587	0	0.000565492
Chitinophagac	0.041722259	0	0.029429815	0.00713284	0.019582792
Rhodospirillace	0.000389148	0.00239521	0.004770141	0.033874946	0.002555188
Others	0.203218813	0.489820359	0.200234323	0.344383191	0.05303062
	1	1	1	1	1

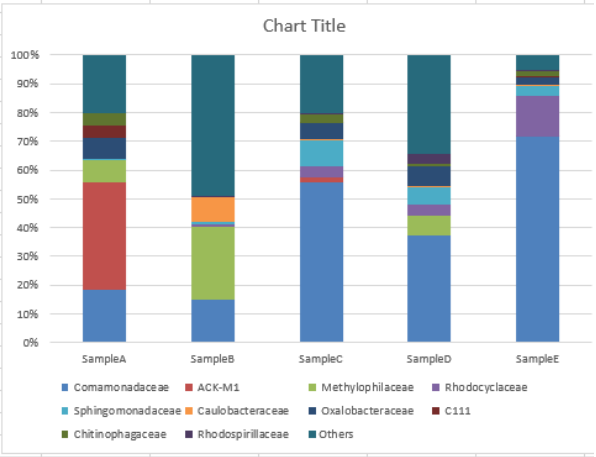

Figure 1: 16S rRNA sequencing data analysis

In Kyoto University

On 14 November, Professor Shogo showed me how to co-culture duckweed callus and agrobacterium as shown in Fig. 2


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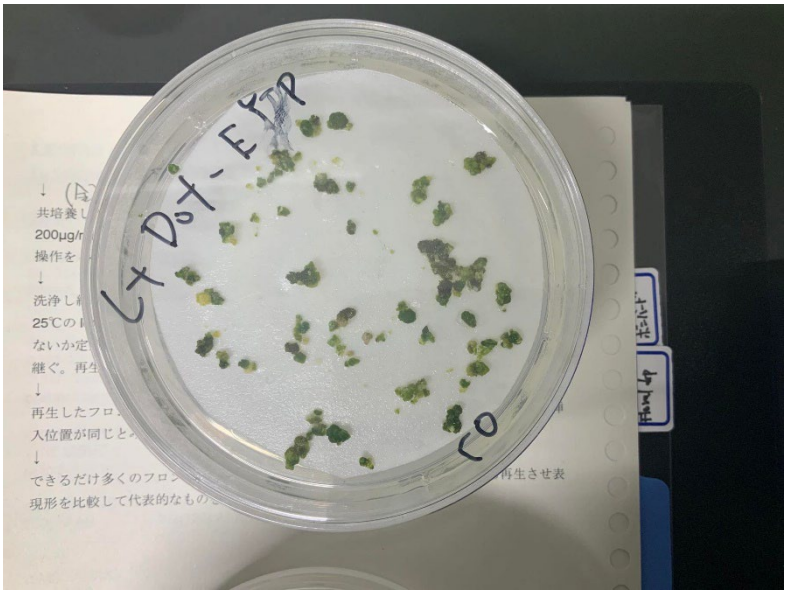

Figure 2: Duckweed callus and agrobacterium co-culture

On 15 November, Prof. Oyama took me a tour for his laboratory and the university.

On 16 November, we continue washing and putting callus on a selective medium plate as shown in Fig. 3

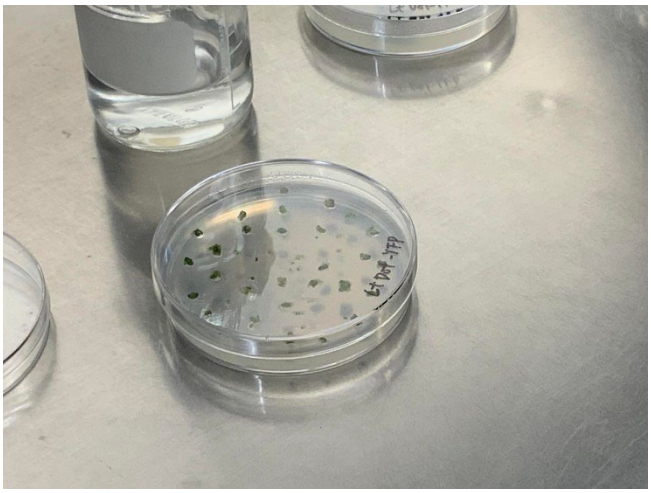

Figure 3: Transformed callus on selective media

On 18 November, We did cryopreservation experiment for *Lemna* and *Spriodela* as shown in Fig. 4.

Figure 4: Cryopreservation on a small aluminum plate.

On 21 November, Dr. Isoda and I did genome DNA isolation for whole genome sequencing by using a kit for high molecular weight

DNA. And also, a master student showed me his paraffin embedding process to observe duckweed structure as shown in Fig. 5.

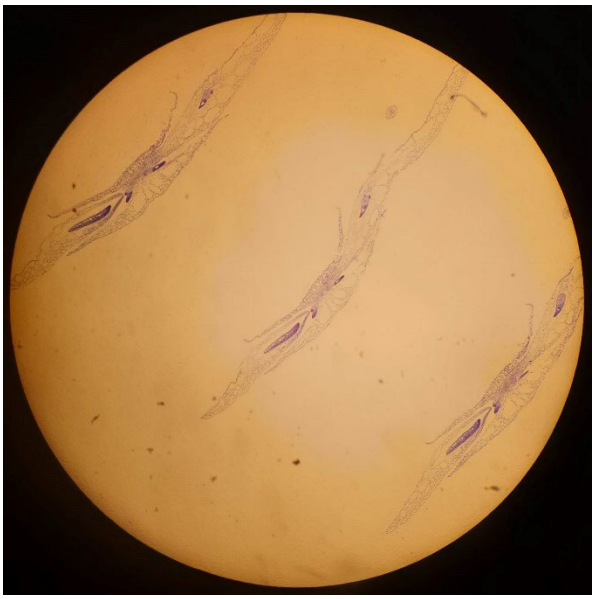

Figure 5: Duckweed structure in paraffin embedding section.

On 24 November, Prof. Oyama and I had a talk about our lab and possible collaboration.

Shogo sensei sent me a photo of cryopreservation result in Fig. 6 and 7. For *Lemna*, it depends on strain as we observe that a strain of *Lemna* showed low percentage of recovery (25%) after cryopreservation but the other strain showed high percentage of recovery (91.7%) after cryopreservation. While *Spirodela* is easy to recover (100%).

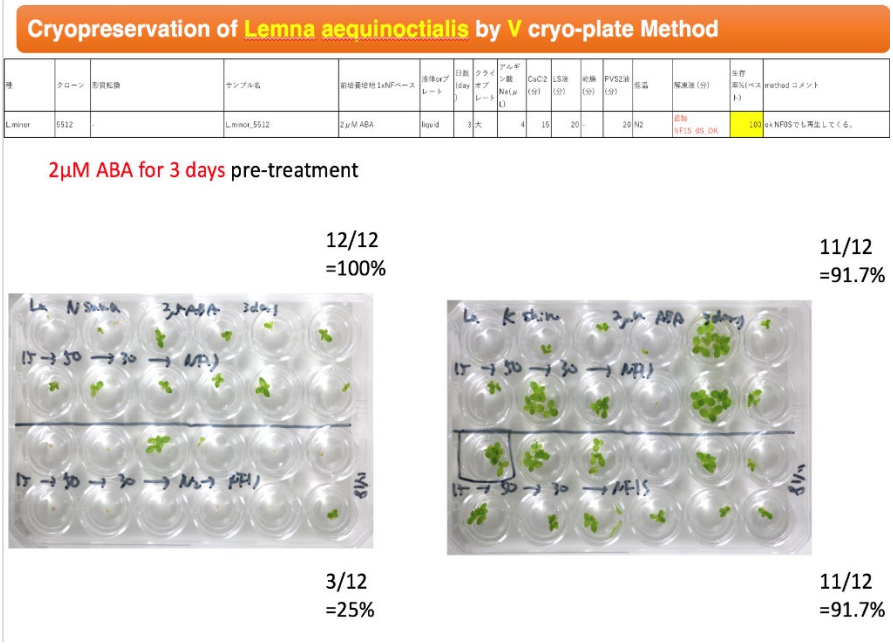

Figure 6: Two strains of *Lemna* recovery after cryopreservation.

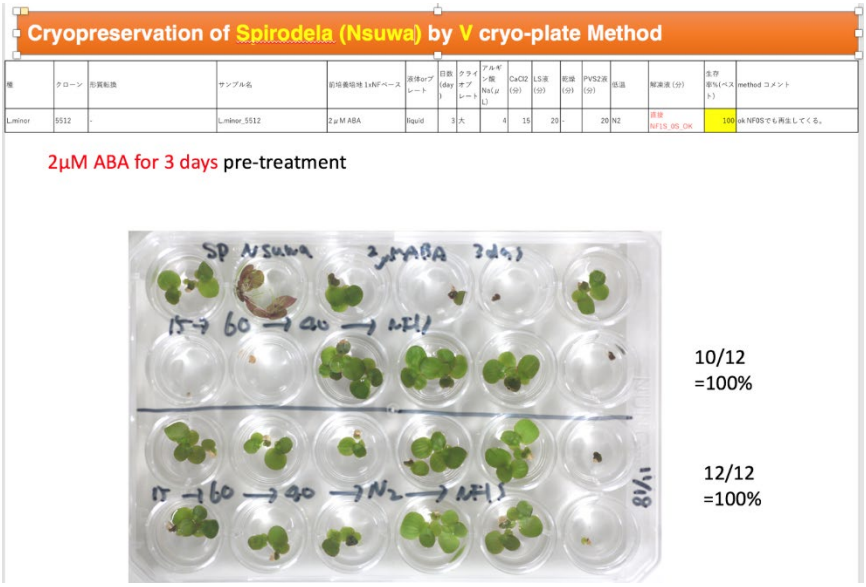

Figure 7: *Spirodela* recovery after cryopreservation.

Conclusion

This training is very useful for me and Thai team. I will definitely use these techniques and knowledge for duckweed research in Thailand and also for duckweed collection for our DHbRC center. I would like to thank Prof. Ike, Prof. Oyama and their lab members for training.

Other than the research aspect, I am impressed with the nice Japan culture, cities and also Japanese people. I am sure that not only research and training I definitely received but also culture and understanding Japanese thought.