SATREPS
Science and Technology Research Partnership for Sustainable Development Program

JICA

Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy

Project Monitoring Sheet

Be-HoBiD

1

Project Monitoring Sheet I

Be-HoBiD

Project Title: Development of the Duckweed Holobiont Resource Values towards Thailand BCG Economy

Japanese side: **Hokkaido U**, Tohoku U, U Yamanashi, Kyoto U, Osaka U, National Institute for Environmental Studies (NIES), Saraya Co. Ltd.

Thai side: **Kasetsart U**, Mahidol U, Khon Kaen U, Chulalongkorn U, Nakhon Pathom Rajabhat U, NBT, BIOTEC, NANOTEC, NSTDA, ADGreen

Period of Project: October 2021 - September 2026 (5-year)

2

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Overall Goal			
Research activities at the Duckweed Holobiont Resource & Research Center (DHbRC) are continued and further developed, and the research activities will be applied to biological resources other than duckweed, so that the project's contribution to the Bio-Circular-Green (BCG) 2 economy will be recognized.	1. Number of cases where DHbRC provided host organisms, associated microorganisms (groups), DNA sequence data, and related information (at least XX cases related to duckweed) (at least YY cases other than duckweed)	>DHbRC and related organizations' materials >Interviews with DHbRC and related organizations	
	2. Number of technologies and cases applied socially by DHbRC and related organizations (at least XX)		
	3. Amount of R&D funding (budget) and project funding (budget) acquired by DHbRC and related organizations (XX THB)		2021 2M THB (KURDI), 2022 4,559,100 THB (KURDI), 400,000 JPY (Kurita Water and Environment Fund)

3

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Project Purpose			
A research development base is established for the development of duckweed industrial technology and its practical use which contributes to Thailand BCG economy.	1. Number of human resources trained (at least XX)	>Project report >Monitoring report >Interview, survey and materials from related entities	1 Director, 1 Vice Director, 1 Manager, 1 Post-doc
	2. Number of valuables developed using duckweed holobiont resources (at least XX)		
	3. Number of entities (universities, government agencies, companies) and a number of individual farmers for which DHbRC provided duckweed, associated microorganisms, DNA sequence data, and related information (at least XX entities, at least XX individuals).		
	4. Number of technical manuals and proposals to promote the duckweed industry (at least XX)		

4

5

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
Outputs			
1. Duckweed Holobiont Resource & Research Center (DHbRC) is established at Kasetsart University.	1. Establishment of DHbRC	>Project report >Established DHbRC >DHbRC's homepage >Constructed duckweed plant factory in DHbRC >Interviews with DHbRC and research groups	DHbRC laboratory and office are established except equipment
	2. Construction of a duckweed plant factory in DHbRC		
	3. Quantity of produced duckweed holobiont biomass to supply to the research groups (adequate amount for research requirement)		
	4. Establishment of functions and services of DHbRC as a common laboratory		

6

7

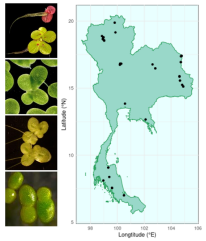
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
2. Duckweed holobiont collection is created	1. Number of preserved individual duckweeds, associated microorganisms, associated microbial communities, and their DNA sequence data (at least 20 plant specimens, 400 microbial strains, 20 microbial communities, 400 DNA sequence data)	>Project report >Created catalog >Published academic papers related to the project	Microorganisms from natural duckweeds: 731 bacteria, 209 actinobacteria, 50 yeasts. Microorganism from wastewater-enriched duckweeds: 131 bacteria, 15 actinobacteria
	2. Creation of a catalog of preserved organisms and relevant information		
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least XX)		1 Publication (IJSEM), 1 Manuscript (in prep)

8

G2 Biodiversity of Thai Duckweed Plants

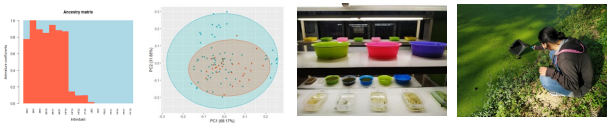
Ekaphan Kraichak
Group PI

Athita Senayai
PhD Student



Research Plan

Topics	2020		2021		2022		2023	
Collection	x	x	x	x	x	x	x	x
Morphology		x	x	x	x			
Ecology		x	x	x				
Genetics	x	x	x	x	x			
Phytochemicals						x	x	x



2020-2022 Output

- Funding from KURDI FF(KU)4.64
- ~ 126 DNA sequences
- Ecological data from 10 sites
- Morphology from 42 plants
- 1 prepared MS
- 2 Bachelor's Thesis
- 1 National Conference Award
- 1 International Scholarship

9

Group 2: Duckweed-microbes holobionts
Biodiversity of duckweed-associated microbes

Assoc. Prof. Dr. Kannika Duangmal
(PI; Actinobacteria)
Dept. Microbiology, Fac. Science, KU

Assoc. Prof. Dr. Nantana Srisuk
(Yeast)
Dept. Microbiology, Fac. Science, KU

Assist. Prof. Dr. Chanita Boonmak
(Bacteria)
Dept. Microbiology, Fac. Science, KU

Dr. Pannida Khunnamwong
(Yeast)
Dept. Microbiology, Fac. Science, KU

Research plan
1. To isolate and preserve associated microorganisms
2. To characterize associated microorganisms
3. To analyze the microbial community associated with duckweeds
4. To create associated microorganism's catalog

Current output
1. Microbial strains (bacteria 731, actinobacteria 209, yeast 50) were isolated from natural duckweed.
2. Bacteria 131 strains, actinobacteria 15 strains were isolated from wastewater-enriched duckweed.
3. Bacteria 176 isolates and actinobacteria 65 isolates were identified using 16S rRNA gene, and yeast 50 isolates were identified using D1/D2 domain.
4. Publication: Saimee, Y. and Duangmal, K. 2021. *Streptomyces spirodelae* sp. nov., isolated from duckweed. Int. Syst. Evol. Microbiol. 71 (11): 005106.
5. Three Master students and two Research assistance (MS)

Future plan: Samples collection (NE, E), isolation and characterization of plant growth promoting traits of isolated microorganisms.

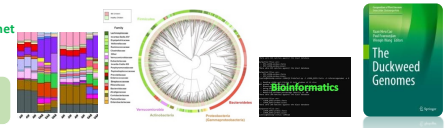
10

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
3. Technology base is developed for function enhancement of the duckweed holobiont.	1. Number of potential duckweed holobionts for function enhancement and wastewater treatment (at least 10)	>Project report >Published academic papers related to the project	1 microbe
	2. Number of omics 6 databases of duckweed and associated microorganisms at DHbRC (at least 5)		2 metagenome data, 1 duckweed genome data
	3. Number of analyses of duckweed and associated microorganisms' interaction (at least 2)		
	4. Number of technologies developed for improvement of duckweed holobionts for growth/stress tolerance (at least 2)		
	5. Number of active substances discovered/identified (at least 5)		
	6. Number of academic papers (including peer-reviewed conference proceedings) published (at least 10)		1 Manuscript (in prep)

11

G3-1: Duckweed-Microbe Interaction Outline

PI
Prof. Dr. Arinthip Thamchaipenet
Genetics, Science, KU



Sample collection
- Natural water, WW from food industry (QP), Animal farm

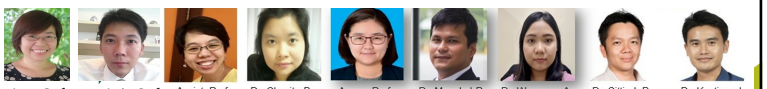
Duckweed Microbiomes
- Metagenome analysis

Duckweed genome analysis

Isolation and characterization of duckweed associated microbes from WW

Selection of duckweed-microbe interaction for WW treatment

Transcriptome and proteome analyses towards growth, nutrition, stress, and WW treatment



Assoc. Prof. Piyada J. | Assist. Prof. Peerapat R. | Assist. Prof. Passorn W. | Dr. Chanita B. | Assoc. Prof. Wanwipa V. | Dr. Mongkol P. | Dr. Waraporn A. | Dr. Sittirak R. | Dr. Kantinan L.

12

Faculty of SCIENCE

G3-2 Active Substances

Department of Chemistry


Witcha Imaram (PI)


Pakorn Wattana-Amorn


Pitak Chuawong


Wanchai Pluempanupat

Research Plan

- Identification of chemical components in the crude extract of each duckweed genus by high resolution mass spectrometry (LC-qTOF)
- Analyze and compare the metabolite composition of duckweed grown under different stimulant conditions.

Outputs

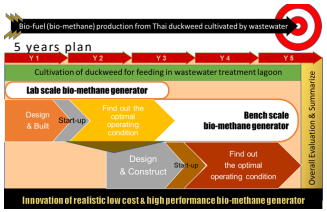
- Two M.Sc. and one Ph.D. Students
- Fundamental Fund, KURDI, 2022 = 500,000 THB
- KU Graduate School Scholarship, 2021 = 200,000 THB (2y).

13

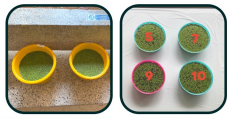
Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
4. Technology base is developed for manufacturing valuables using duckweed as a raw material.	1. Number of technologies developed for manufacturing valuables using duckweed as a raw material (at least XX)	>Project report >Published academic journals related to the project	1 Formula of bioplastics
	2. Number of products of verified selected valuables at the laboratory level or bench plant scale (at least XX)		
	3. Number of academic papers (including peer-reviewed conference proceedings) published (at least XX)		2 Publication (Int J Biol Macromol, Foods), 1 Manuscript (in prep), 2 Proceedings

14

G 4 - 1 Biofuel: Methane

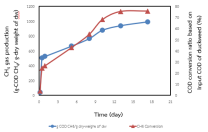


Influent and effluent of KKU Sewage Treatment Pond (STP) and Wastewater from post treatment pond of Tapioca Starch factory were used for cultivation of duckweed. The changes of COD, N, P, pH, temperature were analyzed.


Influent and effluent pond KKU STP

Effluent pond TSW

CH_4 gas production evaluation was done.



Cultivation of duckweed is continuing.
Harvest 500 g wet weight spirodela duckweed/week.

The lab-scale methane fermenters are designed and now in the process of the export from Japan to Thailand.

1 International Conference :
Zhafirah Meuthia Ardhana, Pairaya Choeisai, Krit Choeisai and Kengo Kubota (2022) Growth Rate of Spirodela Cultivated in Sewage Media under the Ambient Conditions of Khon Kaen, Thailand. In Proceeding of the 11th International Conference on Environmental Engineering, Science and Management, Environmental Engineering Association of Thailand, May 12, 2022, Pathumwan Princess Hotel, Bangkok, Thailand, 12R4-01.

15

G4-2: Bioplastics from duckweed biomass

PACKAGING & MATERIALS TECHNOLOGY AGRO-INDUSTRY

GOALS: 1. To add the value and expand the applications in bioplastics of duckweed (DW) biomass

2. To reduce the cost of bioplastics by replacing with renewable low-cost DW materials which has little impact on the food chain

Year 1 | Year 2 | Year 3 | Year 4 | Year 5

Basic research → Technology development

Research to feasibility (proof of concept) → Prototype development

1 Fabrication of DW-based bioplastics with improved performances

Duckweed biomass (DW)
- Municipal and agricultural wastewater stream-treated DW
- Low cost cultivation DW

Renewable and sustainable feedstock for bioplastics

2 Investigation of the relationship between the composition and properties of the obtained DW-based bioplastics

+ Other components

Technologies:
- Direct plasticization
- Blending with other bioplastics and/or commodity plastics

DW-based bioplastic resins/pellets

Possible plastic converting processes
- Injection molding
- Compression molding
- Sheet extrusion & thermoforming

3 Preparation of product prototype of DW-based bioplastics

DW-based bioplastic prototypes/ products

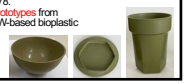
4 Evaluation of preliminary cost of DW-based bioplastics


PI:
Assoc. Prof. Rangrong Yoksan
Department of Packaging and Materials Technology, Faculty of Agro-Industry, Kasetsart University


RA:
Dr. Dang Minh Khanh (Post-doc)


RA:
Miss Apinya Boontanimitr (Post-master)

Outputs:
1. Publication: Yoksan R, Boontanimitr A, Klompong N, Phothongsurakun T. 2022. Poly(lactic acid)/ thermoplastic cassava starch blends filled with duckweed biomass. Int J Biol Macromol 203: 369–378.
2. Prototypes from DW-based bioplastic



16

Group: G4-3 Animal feed

Researcher team


Asst. Prof. Dr. Chanwit Kaewtapee
Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok


Asst. Prof. Dr. Sirinapa Chungopast
Department of Soil Science, Faculty of Agriculture, Kasetsart University, Nakhon Pathom


Ms. Hathaipat Thongthung
(Master Degree Student in Animal Nutrition)
Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok


Dr. Nattika Saengkrit
National Nanotechnology Center National Science and Technology Development Agency


Dr. Kantinan Leetanasaksakul
National Center for Genetic Engineering and Biotechnology
National Science and Technology Development Agency

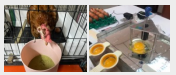
Research plan and out put



To study *In vitro* / *In vivo* digestibility


To isolate protein and encapsulate it release at lower GI-tract.


To study gut microbiome.


To study effect of duckweed on production performance and egg quality


To analysis nutrient compositions

17

G4-4 Functional food: Impact of active-enriched components from duckweed (*Wolffia globosa*) on gastrointestinal digestibility and human gut microbiome modulation

Asst. Prof. Dr. Suvimol Charoensiddhi (Dept. Food Science and Technology, Fac. Agro-industry, KU)**- PI**
Assoc. Prof. Dr. Wanwipa Vongsangnak (Dept. Zoology, Fac. Science, KU)**- Member**
Assoc. Prof. Dr. Massalin Nakphaichit (Dept. Biotechnology, Fac. Agro-Industry, KU)**- Member**
Dr. Mongkol Pongsuchart (Dept. Zoology, Fac. Science, KU)**- Member**
Asst. Prof. Narissara Suratannon, M.D. (Dept. Pediatrics, Fac. Medicine, CU)**- Member**

Aims & Plan:
1. **Active-enriched components from duckweed leading to health benefits is developed.**
2. **Functional food applications are produced from duckweed active-enriched components.**

Y1	Y2	Y3	Y4	Y5
1.1 **Collection and preparation** of duckweed **raw materials** 1.2 Development of **production processes** for duckweed active-enriched components	1.3 Investigation of **digestibility** of active-enriched components in simulated human digestive system 1.4 Assessment of **health benefits** 1.5 **Characterization** of major compositions	1.4 & 1.5 Continue 2.1 Development of **up-scale production process** for active-enriched components 2.2 Study of **physicochemical and functional properties** in food model	2.2 Continue 2.3 Assessment of **acute and subchronic toxicity** of active-enriched components in an *In vivo* model	2.4 Development of **functional food applications**

Key output: The optimum condition to extract protein-enriched fractions from dried duckweed raw material was achieved. 1 Proceedings & 1 Publication

18

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
5. Low-carbon effect of the water purification system using duckweed holobiont is verified.	1. Energy saving rate of the entire system (Energy consumption lower than the Business-As-Usual (BAU))	>Project report >Created database/handbook >Published academic journals related to the project	
	2. Amount of green-house-gas (GHG) emission reduction (GHG emission lower than the BAU)		
	3. Database/handbook for design and operation management of duckweed holobiont water purification system		
	4. Number of academic papers (including peer-reviewed conference proceedings) published (at least 5)		1 Book Chapter (in prep)

19

【G5-Duckweed for wastewater treatment】

Development of prototype duckweed wastewater treatment with co-benefit in terms of duckweed biomass production and greenhouse gas mitigation

Research team: Dr. Chart Chiemchaisri (PI), Dr. Wilai Chiemchaisri (researcher) Dr. Noppharit Sutthasil (post-doc), Ms. Chonnada Chandaravithoon (PhD student), Ms. Laksika Saksukol (Master student)

Research plan (Year 1):

1) Survey of wastewater sources, water qualities and preparation of selected sites
2) Bench (lab) scale investigation for selection of appropriate duckweed species and optimum operating conditions of duckweed from farm wastewater and factory influent/effluent

Current output:

1) Pig farm and Kewpie factory were identified as wastewater sources. Their wastewater characteristics were analyzed.
2) Lab-scale experiments to determine duckweed growth and wastewater characteristic changes were performed. Duckweed species which grew well in both wastewaters were identified. The duckweed growth and wastewater purification (COD, N) during treatment in batch reactor was studied.
3) A book chapter entitled "Duckweed based waste stabilization ponds for wastewater treatment" is under preparation for "Low Cost Water and Wastewater Treatment Systems: Conventional and Recent Advances" book to be published by Elsevier.

20

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Achievement
6. Support for duckweed production in farms and practical application of technology using duckweed are promoted.	1. Number of publication/dissemination/public relations activities of results from each research group (at least XX)	>Project report	
	2. Number of support activities for duckweed production (at least XX)	>Dissemination and public relations activity reports >Created technical manuals and proposals	
	3. Number of created technical manuals and proposals of technology using duckweed (at least XX)		

21

Be-HoBiD Output 2022

Publications:

1. Saimee, Y. and **Duangmal, K**. 2021. Streptomyces spirodelae sp. nov. isolated from duckweed. **IJSEM**, 71 (11): 005106.
2. **Yoksan R**, Boontanimitr A, Klompong N, Phothongsurakun T 2022. Poly(lactic acid)/ thermoplastic cassava starch blends filled with duckweed biomass. **Int J Biol Macromol** 203: 369–378.
3. Duangjarus N, Chaiworapuek W, Chitsiri R, Ritthiruangdej P, **Charoensiddhi S**. 2022. Antimicrobial and functional properties of duckweed (*Wolffia globosa*) protein and peptide extracts prepared by ultrasound-assisted extraction. **Foods**, 11, 2348.

Manuscript (in prep):

1. Senayai, A., **Kraichak, E.** 2022. Genetic and morphological variation among populations of duckweed species found in Thailand. **Plants**. (in prep).
2. Bunyoo C, Roongsattham P, Khumwan S, Phonmakham J, Wonnapinij P, **Thamchaipenet A**. 2022. Microbial communities of duckweeds in nature and in nutrient deficient condition. **Plants** (Submitted)
3. **Yoksan R**, Boontanimitr A 2022. Effect of calcium carbonate and titanium dioxide on properties of biodegradable polyesters filled with duckweed biomass. (in prep)
4. **Chiemchaisri C**, Chiemchaisri W, Sutthasil N, Chandaravithoon C, Saksukol L 2022. Duckweed based waste stabilization ponds for wastewater treatment. In "Low Cost Water and Wastewater Treatment Systems: Conventional and Recent Advances", Elsevier (in prep).

22

Be-HoBiD Output 2022

Conference:

1. Senayai, A., **Kraichak, E.** 2022. Population genetic structure and morphology of Duckweed species in Thailand. **The 14th Botanical Conference of Thailand**, Khon Kaen University, 11-12 June 2022
2. Bunyoo C, Roongsattham P, Khumwan S, Phonmakham J, Wonnapinij P, **Thamchaipenet A.** 2022. Microbial communities associated with duckweeds in nature and in nutrient-deficient condition. **International Conference of Genetics Society of Thaland (iGST2022)**, 21-22 June 2022.
3. Ardhana ZM, **Choeisai P**, Choeisai K, Kubota K. 2022. Growth rate of Spirodela cultivated in sewage media under the ambient conditions of Khon Kaen, Thailand. In **Proceeding of the 11th International Conference on Environmental Engineering, Science and Management, Environmental Engineering Association of Thailand**, 12 May 2022
4. Duangjarus N, Chaiworapuek W, Ritthiruangdej P, Sae-tan S, **Charoensiddhi S**. 2021. Ultrasound-assisted extraction for the recovery of proteins from duckweed (*Wolffia globose*) using response surface methodology. **The Proceedings of the 59th KU Annual Conference**, 2. 655-662.

No. of postgrad students & RA: 13 MS, 5 PhD, 2 Post-doc, 7 RA

23

Be-HoBiD Output 2022

Research grant:

1. Fundamental Fund, KURDI — 2021 = **3M** THB; 2022 = **4,574,100** THB
2. Kurita Water and Environment Fund — 2022 = **400,000** JPY

Scholarship:

1. Canada-ASEAN scholarships and Educational Exchange for Development (SEED), EduCanada (Ph.D)
2. Post-Master Scholarship, KU Reinventing U, NRCT, 2021 = **600,000** THB (2x)
3. KU Graduate School Scholarship, 2021 = **400,000** THB (2y) (2x)
4. KU Graduate School Scholarship, 2022 = **300,000** THB (3y)
5. Doctoral Scholarship from Faculty of Engineering, KU, 2022 = **786,000** THB (3y);
6. KKU scholarship for ASEAN & GMS Countries' Personnel for Master student, 2021 = **228,000** THB (2y)

Awards: Outstanding Presentation Award from **14th Botanical Conference of Thailand**

24