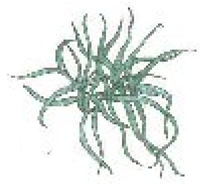
ISCDRA



International Steering Committee on Duckweed Research and Applications

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More information about the ISCDRA and previous issues are available at http://lemnapedia.org/wiki/ISCDRA

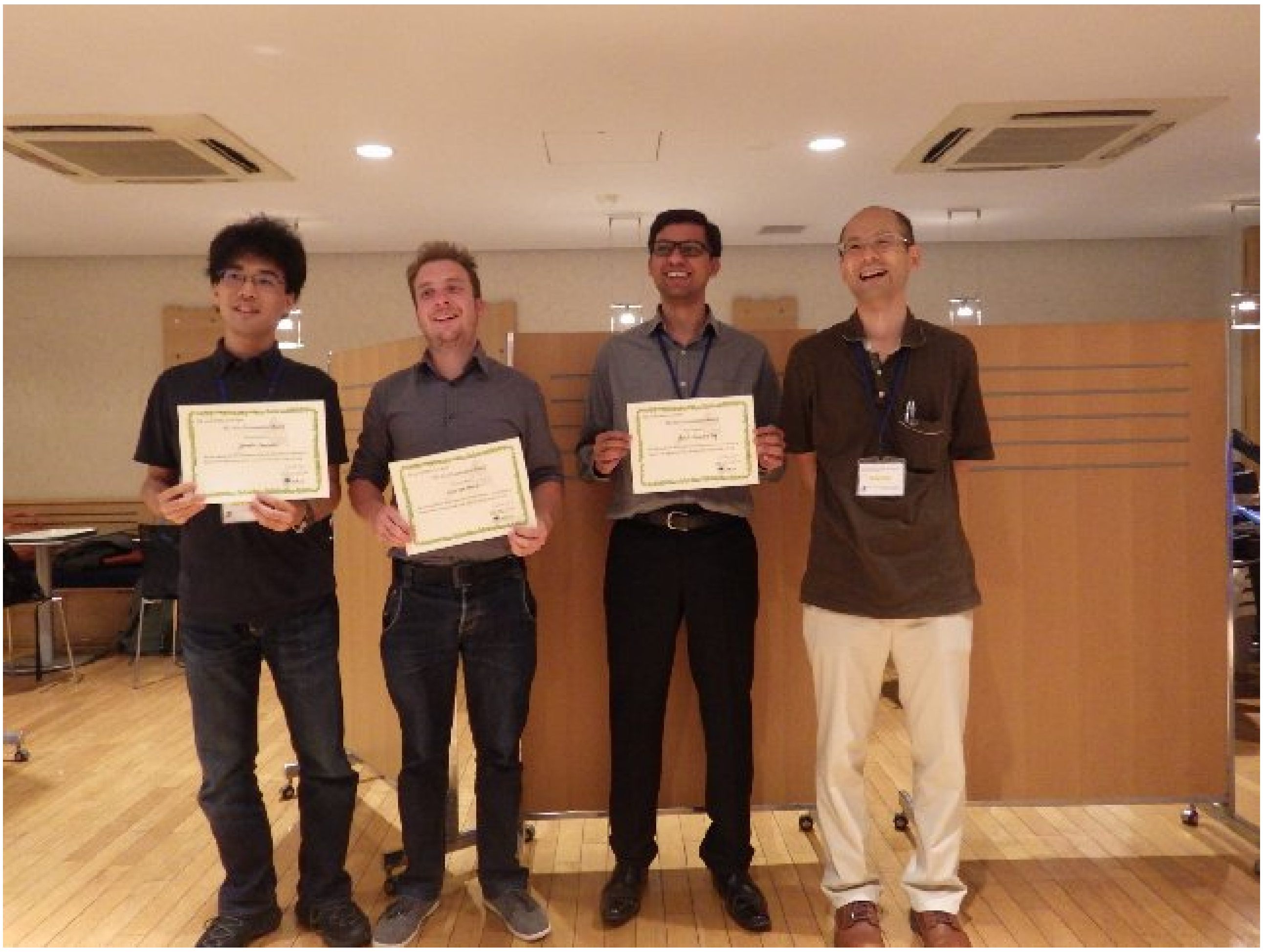
Letter from the Editor

Dear Friends of Duckweed,

The much-anticipated 3rd International Conference on Duckweed Research and Applications has been successfully completed on July 6th of this year in Kyoto.

We decided to inform all interested people on some of the key outcomes of this meeting by preparing a special issue of the ISCDRA newsletter. In it you will find the abstracts of oral and poster presentations, hopefully giving you a good overview of the conference proceedings. Some of the highlights include the announcement of the complete genomic sequence of *Spirodela polyrhiza* 9509 resolved at the chromosome level using high-throughput methods, update on sequencing of two different *Lemna minor* clones (5500 and 8627) and one clone of *Lemna gibba* (7742a, better known as G3). In addition, we were given an update on the genetic transformation of Lemna. The technique mcFISH was used to test and improve the structure of the *S. polyrhiza* 7498 genome draft.

Much attention has been given to the interaction of several Lemna species and microorganisms (bacteria and fungi) in order to improve the growth of duckweeds for special applications. From the complete genome sequence of one such PGPB, a novel cluster of genes has now been demonstrated to be enough and necessary for the production of the plant growth-promoting molecules. This exciting demonstration as well as several other papers described results that are likely to encourage development of biotechnological applications.



On the final day of the Conference, three young presenters, selected by a committee of senior investigators invited from among the participants, were honored with certificates for their excellent presentations by the organizer, Prof. Tokitaka Oyama (shown on the right). The award winners are (from left to right): Tomoaki Muranaka (P9, Kyoto University), Arne Van Hoeck (L3, University of Antwerp) and Jog Rahul (L12, Hokkaido University).

And there is more – just read the abstracts. Additionally, there are news related to the installation of new members for the ISCDRA team and the announcement of the next venue for our 4th ICDRA in India in 2017.

Best wishes to all of you.

On behalf of the Steering Committee

Klaus-J. Appenroth

ISCDRA elections and new term

This is the new *International Steering Committee on Duckweed Research and Applications*. The Committee was elected in advance of the 3rd Conference by voting open to all past and present ICDRA attendees via Internet.

The Head was re-elected during the time of the meeting by the members of the Committee.

Eric Lam	Tamra Fakhoorian	Klaus Appenroth Head	Louis Landesman	Eduardo Mercovich

Commercial Applications Perspective on Conference Findings

Tamra Lynn Fakhoorian, International Lemna Association, ISCDRA Committee, GreenSun Products, LLC

I attended the 3rd ICDRA with great anticipation, being on the lookout for research that could be applied to the nascent duckweed industry. While there was a strong focus on genomics, (long term applications) there have been many developments in the field of how bacteria and duckweed interrelate (short term applications). This synergy is of high interest as growers are looking for more efficient means to produce stable, consistent production with high protein and starch yields.

Here are just some of the highlights that apply to commercial production:

- The use of select plant growth promoting bacteria (PGPB) were found to produce a short run in a greenhouse setting of the equivalent of 38 t/ha/yr high starch yields by Wolffia. (L7)
- PGPB-induced accelerated growth increased chlorophylls and enhanced photosynthesis of *Spirodela polyrhiza*. (L9)
- PGPB was used to speed plant growth of *Lemna minor* in wastewater setting by 30% times over the control. (L10)

While the mechanisms and pathways are still unclear, some advances are being done, like the identification of a P23 gene cluster responsible for plant growth promotion, in Dr. Morikawa's lab. Bacterial synergies with duckweed species are a very promising approach to optimizing production on a commercial scale. Another study of interest regarded improved production in the presence of yeast mannan in growth trials.

Remediation and mitigation studies involving duckweed offer new opportunities for companies looking for solutions to the following scenarios:

- Selenium remediation via *L. punctata* yielding a high Se content biomass useful as an antioxidative food source for aquaculture. (L20)
- Methane mitigation via C-1 microbes on duckweed fronds in natural systems. (L24)
- Estrogen degradation by *Rhodococcus zopfii* Y 50158 and *Lemna minor* in wastewater settings on an as yet, limited basis. (L26)

Phytohormone studies showed that growth hormones for terrestrial plants affected *Lemna minor* differently. While several plant hormones inhibited growth, both Fe-EDTA and indole 3-acetic acid (auxin) were found to increase plant growth at various minimum and maximum concentrations. (P5)

The Sri Lankan fisheries study involved duckweed powder at a 10 and 20% basal feed inclusion rate proved successful with tilapia fry. (L22)

ICDRA 2015 abstracts

Presentations abstracts

L1. Omics and genetic transformation research of duckweed

Hai Zhao

Chengdu Institute of Biology, Chinese Academy of Science

On the mechanism research of starch accumulation for duckweed, we established the protein library our own by the transcriptome data, which greatly improved the recognition rate of protein. We systematically elaborated the starch accumulation mechanism of duckweed under nutrition starvation from the 4 level of physiology, biochemistry, transcriptome and proteome. By testing the total flavonoid content, comparing the component change of flavonoid, transcriptome and proteome analysis of flavonoid and ligin synthesis pathway, this research find out the mechanism of low ligin content and high flavonoid content in duckweed.

We found exogenous hormone Uniconazole could significantly raise the starch content and yield of duckweed. By comprehensive analysed the hormone content, transcriptome and proteome data of hormone synthesis pathway, our research explained the influence of Uniconazole to hormone synthesis in duckweed. Systematically elaborated the starch accumulation mechanism of duckweed under Uniconazole treatment from the 4 level of physiology, biochemistry, transcriptome and proteome. By further analyzing on hormone receptor and expression of transcription factor form transcriptome level, combined with the reporte in the literature, find out the interaction of hormone cause the starch accumulation.

The period of duckweed genetic transformation by callus is at least 12 months. Using fronds directly for genetic transformation can get the transgenic plant in 3-4 month. We have already eatablished a genetic transformation method of duckweed fronds mediated by *grobacterium tumefacien*. Therefore duckweed can be used to bioreactor for oral vaccine, and a kind of model plant for functional gene research.

L2. Genetic engineering of Lemnaceae: tools for research and applications

A. Mollá-Morales[1], A. Cantó-Pastor[1], E. Ernst[1], W. Dahl[1], J. Zhai[2], Y. Yan[3], B. C. Meyers[2], J. Shanklin[3] & R. Martienssen[1,4]

[1] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

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[4] Howard Hughes Medical Institute – Gordon and Betty Moore Foundation, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

Lemnaceae are the smallest aquatic flowering plants. Their robust growth in marginal environments, their particular metabolic traits, such as high starch accumulation and low lignin content, and their simple architecture, make them a great system for research and commercial applications.

The capability of genetic engineering of Lemnaceae would increase its value as a model system and would open a door to generate high value products (it has already been used to produce recombinant proteins and human monoclonal antibodies), but the low transformation efficiency methods which currently exist have held back efforts to perform major genetic studies.

We have developed a fast and efficient method for producing Lemna minor stable transgenic fronds via Agrobacterium-mediated transformation and regeneration from tissue culture. Transgenes can be used for the overexpression of genes but strategies for silencing need to be tested on Lemnaceae. We analyzed the Lemna gibba genome looking for possible micro RNA (miRNA) precursors, and found several of them. We used these precursors to construct artificial miRNA (amiRNA) targeting a gene whose silencing should cause a visual phenotype. The results obtained showed that posttranscriptional gene silencing (PTGS) via amiRNA is not only possible but also highly specific.

L3. Sequencing, assembling and annotating Lemna minor genome for use in transcriptomic analyses

Van Hoeck Arne, Horemans Nele, Van Hees May, Nauts Robin, Vandenhove Hildegarde, Blust Ronny

SCK CEN and University of Antwerp (Belgium)

Lemna species have a number of attributes that made them advantageous for use in laboratory toxicity testing. They are relatively small and have a rapid vegetative growth forming genetically uniform clones, eliminating potential effects due to genetic variability. *Lemna sp*. have proven their value in ecotoxicological research as representatives of higher aquatic plants with standardized guidelines on how to perform a growth inhibition test (OECD guideline 221). To unravel the toxic mode of environmental stress responses in plants, additional mechanistical knowledge is needed. This information could potentially be retrieved from molecular fingerprints. Although the genome of *Spirodela* has been published last year, no genetic information for *Lemna sp*. is available.

In an effort to strengthen duckweed genomic research, we present a draft genome of *Lemna minor*. Different algorithms for genome assembly were evaluated like SOAPdenovo, CLC bio and Masurca. The latter approach generated the least amount of contigs, contributing to an N50 value of 19200 bp. This 479 Mbp long sequence was subsequently subjected to gene recognition programs in order to locate gene loci using transcript evidence from RNAseq data and protein evidence from *Spirodela* and 8 other molecular characterized monocotyl plants. As such 18784 predicted protein-coding genes were identified which is comparable to the *Spirodela polyrhiza* genome containing of 19623 protein-coding genes. Using InterProScan, 12694 could be annotated and received a Gene Ontology accession number from the GO database whereas 8337 genes (45%) were linked to a biological function.

The here developed *Lemna minor* draft genome can be used as a reference genome for transcriptomic analysis. As an example, RNAseq studies were performed to analyze the biological effects and interactions between different radiation types in *Lemna minor*. RNA was extracted from plants exposed to uranium, β-radiation and γ-radiation. It was found that the three different stress conditions had 17 differentially expressed genes in common. A comparison between the molecular fingerprints of the different radiation types will be further presented. We can conclude that the draft genome is a reliable reference sequence to assist future RNAseq studies in *Lemna minor.*

The presentation is available at
http://lemnapedia.org/wiki/Sequencing,_assembling_and_annotating_Lemna_minor_genome_for_use_in_transcriptomic_analyses

L5. Fast forward approach to a high-resolution genome sequence map and quantification of intraspecific structural and sequence variations in Spirodela polyrhiza strains.

Eric Lam[1], Doug Bryant[2], Ryan Gutierrez[1], Philomena Chu[1], Han Zhong Zhang[1], Kennith Acosta[1], Jing Xia[3], Todd Mockler[4], Weixiong Zhang[3] and Todd Michael[2]

[1]Department of Plant Biology, Rutgers, the State University of New Jersey, New Brunswick, NJ, USA; [2]Ibis Bioscience, Carlsbad, CA, USA; [3]Washington University, St. Louis, MO, USA; [4]Danforth Center, MO, USA

Floating aquatic plants of the duckweed family hold the promise to provide a novel platform for sustainable agriculture. A well-assembled and validated reference genome for duckweed will enable rapid development of molecular tools and resources to realize its potential as a new crop. The Giant Duckweed, *Spirodela polyrhiza,,* has one of the smallest monocot genomes at ~150 Mbp and 20 chromosomes. We deep-sequenced (>100X coverage) *Spirodela* 9509 strain leveraging a mixture of Illumina short-read sequencing libraries. The initial draft assembly covers 92% of the predicted sequence space in 32 super-scaffolds, which is similar to the first draft (~20X) from strain 7498 that was sequenced using 454, fosmids and BAC-ends. Sequence comparison between the genomes of strains 9509 and 7498 revealed over 500,000 SNPs and 70,000 indels. Annotation of single-copy, homologous gene pairs from the two strains provided positional anchors to further collapse the 32 super-scaffolds from each genome draft into larger pseudo-molecules. This strategy of enhanced genome assembly was validated and refined by PCR analyses using selected junctions to yield 23 pseudo-chromosomes. Furthermore, comparative analysis of annotated gene sets from the two genome sequence drafts provided evidence for 10% more gene models than predicted from either assembly alone. To further close gaps in the genome assembly, we leveraged Irys™ (BioNano Genomics) single molecule DNA optical mapping technology to produce genome-wide feature maps (GFMs) for both *Spirodela strains*. We resolved the 9509 genome into its 20 chromosome using 200x GFM coverage. Lastly, comparison of GFMs from the two

strains of *Spirodela* provided the first genome-wide identification of intraspecific structural variations in a plant model. Application of this high-throughput physical mapping technology should lead to accelerated characterization of genome sequence and structure for other duckweed species.

L6. Whole Genome Sequencing of Lemna minor

Evan Ernst, Rob Martienssen

Cold Spring Harbor Laboratory

Lemna minor clone 8627 was among the first duckweeds for which a stable transformation protocol was demonstrated nearly 15 years ago. In subsequent studies, it was found to possess a high growth rate on concentrated wastewater along with a favorable protein production rate. We have assembled an initial draft of the genome sequence from over 75 Gbp of Illumina PCR-free, mate pair and PacBio data, spanning approximately 800 Mbp, the largest duckweed genome sequenced to date. The availability of this genome sequence, together with the development of new transformation protocols and artificial miRNA constructs in *L. minor* enables rapid and precise genetic engineering of this duckweed clone.

L7. Starch production capability of rootless duckweed and its enhancement by plant growth promoting bacteria (PGPB)

Kazuhiro Mori[1], Tadashi Toyama[1], Yasuhiro Tanaka[1], Masaaki Morikawa[2]

[1]Interdisciplinary Graduate School, University of Yamanashi

[2]Graduate School of Environmental Science, Hokkaido University

[Background and Objectives] Rootless duckweed of the genus of *Wolffia* is a small floating aquatic plants found in tropics or subtropics. Vegetative frond of *Wolffia* exhibits high growth rate and protein content. The dormant buds, however, sink underwater spontaneously accumulating abundant starch. In this study, we elucidate the growth characteristics and starch production capabilities of several *Wolffia* plants collected in Japan. Plant growth promoting bacteria (PGPB) for efficient protein production by rootless duckweed is also investigated.

[Methods and Results] Five plants strains (*W. globosa*) were collected in ponds and agricultural wet fields in Japan. *W. arrhiza* kindly given by Prof. E. Landolt of the Swiss Federal Institute for Technology (ETH) and subcultured for 20 years under the germ free condition was also used as a plant material. Plants batch cultivation in laboratory scale was aseptically carried out using Hutner nutrient solution in a green chamber. Middle size continuous open culture in a greenhouse was also performed. PGPBs efficient for rootless duckweeds were isolated from a plant sample cultured in a pond. Six plants material used in this study indicated high growth rate under wide range of illumination, temperature, pH, and nutrient concentration. The starch content was highly affected by nutrient condition and optimal condition induced 50-60% (dry weight) starch contents. Short term continuous cultivation in a greenhouse indicated various but high starch production rate of six plants materials equivalent to 3-8 t-starch ha^{-1} y^{-1}. PGPBs isolated from *Wolffia* sample collected in a pond showed 20-30% growth promotion of the plants materials. Inhibitory effect on the starch production by *Wolffia* was not observed. It can be concluded that rootless duckweeds used in this study are useful plants material exhibiting remarkable starch producing capability in environmental water which are equal with grains. Furthermore, they can be promoted by some proper combination with PGPB materials.

L8. Microbial community structure associated with different duckweed species in different environmental waters

Yan LI, Tadashi TOYAMA, Yasuhiro TANAKA, Kazuhiro MORI

Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Kofu, Yamanashi, Japan

Duckweed is a fast-growing and world-widely spread aquatic plant, which include five different genera. Duckweed-related microbial populations were found to play an important role in promoting plant growth, removing nutrients and degrading organic pollutants. Study on the duckweed-related microbial community will facilitate utilizing them in phytoremediation and biomass production. The objective of this study is to evaluate the effects of duckweed-species and environmental water in shaping the duckweed-related microbial community structure.

Sterilized *Spirodela polyrhiza*, *Lemna minor* and *Wolffia arrhiza* were separately incubated in three environmental water (two river water samples and one secondary effluent sample from sewage treatment plant) for 7 days. Total bacteria attached on the plant surface were collected from the whole plant body (*W. arrhiza*), the roots and leaves of *S. polyrhiza* and *L. minor*, respectively. Total bacterial community compostion was analyzed by pyrosequencing technique.

The results showed that: The microbial communities on the duckweed plant surface were obviously different from that in the original water. The samples from the river water environment showed different microbial community composition from that in secondary effluent water sample. Duckweed can accumulate *Comamonadaceae* and *Rhodocyclaceae* on the plant surface, regardless of the surrounding water environment. It indicated that duckweed plants the main decisive factor in shaping the microbial community structure. Surrounding environmental water also can influence the microbial community composition to a certain degree.

L9. Plant growth-promoting bacteria (PGPB)-induced accelerated growth, increased chlorophylls and enhanced photosynthesis of duckweed (Spirodela polyrrhiza)

Tadashi Toyama, Yasuhiro Tanaka and Kazuhiro Mori

University of Yamanashi

Plant growth-promoting bacteria (PGPB) are beneficial bacteria that colonize plant roots and improve plant growth. However, the mechanisms and pathways involved in the interactions between PGPB and plant are unclear. *Sinorhizobium* sp. SP4 has been isolated from a duckweed species, *Spirodela polyrhiza*. In order to better understanding the complex PGPB-plant interactions, changes in the transcriptome of *S. polyrhiza* co-cultured with strain SP4 were analyzed. Here, we performed RNA-seq transcriptional profiling of *S. polyrrhiza* co-cultured with strain SP4 and sterile *S. polyrhiza* (control).

After 3 days co-culture of *S. polyrhiza* with strain SP4 in Hoagland solution, the rates of biomass (dry weight) growth, photosynthesis activity and chlorophylls *a*/ *b* content of SP4-inoculated *S. polyrhiza* were up to about 2 times higher than those of control *S. polyrhiza*. In addition, we identified over 35,000 *S. polyrrhiza* expressed sequence tags. Some genes encoding proteins related to photosynthesis were highly expressed in *S. polyrrhiza* co-cultured with SP4 compared with control *S. polyrhiza*. Our data will support the use of PGPB for growth promotion in duckweed-based wastewater treatment and bioresource production systems.

L10. Enhanced growth of Lemna minor by Acinetobacter calcoaceticus P23 in environmental water

Masashi Kuroda[1], Yoshiyuki Hachiya[1], Koichiro Tokura[1], Angela Quach[1], Yuka Ogata[2], Tadashi Toyama[3], Masaaki Morikawa[4], and Michihiko Ike[1]

[1]Graduate School of Engineering, Osaka University
[2]Research Center for Material Cycles and Waste Management, National Institute for Environmental Studies
[3]Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, [4]Graduate School of Environmental Science, Hokkaido University

Phytoremediation of eutrophic water bodies using duckweed is an attractive technology due to the low energy input, low cost, and biomass resource production as by-product. Utilization of symbiotic relation between duckweed and beneficial bacteria can improve the performance and productivity of the phytoremediation. Based on the concept, growth promotion of a duckweed, *Lemna minor*, by a plant growth promoting bacterium, *Acinetobacter calcoaceticus* P23, in environmental water was investigated. Strain P23 promoted the growth (both number of fronds and dry weight) of *L. minor* acclimated in pond water by 1.3 times during 14-day cultivation compared with the plant in absence of strain P23. However significant growth promotion was not observed in sterile pond water, suggesting that the strain P23 promoted the growth of *L. minor* in cooperation with certain indigenous bacteria in pond water. In the repeated batch cultivation, strain P23 was maintained on

L. minor surface and promoted its growth during first two batches. However, strain P23 disappeared from the surface of *L. minor* and growth promotion was not observed in the third batch. In the fourth batch after re-inoculation of strain P23, the attachment of a certain amount of P23 on *L. minor* was observed, and the growth was successfully promoted. These results suggest that the growth promotion is strongly associated with the amount of P23 on the plant, and it is important to maintain the amount of P23 on the plant for sustainable growth promotion of *L. minor* in environmental water.

L11. Growth promotion of Wolffia globosa by Acinetobacter calcoaceticus P23

Maiko Sakaguchi[1], Yoshiyuki Hachiya[1], Masashi Kuroda[1], Masaaki Morikawa[2], Michihiko Ike[1]

[1] Graduate School of Engineering, Osaka University
[2] Graduate School of Environmental Science, Hokkaido University

Wolffia globosa, a small duckweed, has high potential as the biomass resource, because it contains much protein and starch. Growth promotion of *W. globosa* by Plant Growth-Promoting Bacterium (PGPB) can contribute to the development of efficient biomass production technologies. In this study, we evaluated the feasibility of *W. globosa* growth promotion by *Acinetobacter calcoaceticus* P23, which is a PGPB associated with duckweeds *Lemna aoukikusa* and *Lemna minor*, under various conditions. P23 promoted growth of *W. globosa* approximately double in the A&H medium and filtered pond water within 14 days. In the medium without ethylenediaminetetraacetic acid (EDTA), inoculation of P23 caused the plant growth comparable to that with EDTA and increase the chlorophyll content, suggesting that P23 increases the availability of metal by providing an alternative chelating agent to enhance the photosynthesis in the plant. To evaluate the influence of indigenous microbes in environmental water, *W. globosa* was cultivated in P23 suspension in pond water filtrated with membrane filters with pore size 1.0 (removing algae or protozoan), 0.65, 0.45(partially removing bacteria) or 0.20 μm (filter sterilization). As a result, P23 significantly promoted the growth in the pond water filtrated with 1.0, 0.65 and 0.45 μm-pore size filters, but not with 0.20 μm-pore size filter. However, the growth promotion was observed in the experimental systems only when accompanied with the decrease of P23 cell density in the liquid phase, which was probably caused by the indigenous bacteria in pond water. These results suggest that P23 promotes the plant growth in association with indigenous bacteria. These results indicated the usefulness of *W. globosa* – P23 interaction to achieve efficient biomass production which may be coupled with purification of eutrophic environmental water.

L12. Molecular mechanisms of duckweed growth-promotion by Acinetobacter calcoaceticus P23

Jog Rahul, Masayuki Sugawara, Kyoko Miwa, Masaaki Morikawa

Graduate School of Environmental Science, Hokkaido University

In our previous study we have shown that *Acinetobacter calcoaceticus* P23, isolated from duckweed of the Botanical Garden, Hokkaido University, significantly promotes duckweed growth and increases its chlorophyll content. It is assumed that bacterial plant growth promoting factors for aquatic vegetation are different from those compared to terrestrial plants. In our present study, we have demonstrated that production and purification of extracellular polysaccharides (EPS), PGF-P23, from P23 strain and proved it to be the key factor responsible for promoting duckweed growth. Furthermore, based on whole genome analysis of P23, we cloned a potential gene cluster, p23_2757-2761, responsible for EPS production in non-plant growth-promoting *Acinetobacter baylyi* ADP1 using broad host range vector and tested its effect on the duckweed growth. Results indicated that P23 possesses a novel gene cluster encoding unique glucosyl and mannosyl transferases responsible for the plant growth promotion. Also, fluorescence staining of *A. baylyi* ADP1 carrying p23_2757-2761 gene cluster indicated increased sugar content in the biofilm as compared to the vector control. Taking all results together, we are allowed to conclude that EPS (PS-I) produced by a set of novel genes in P23 strain have significant duckweed growth promoting activity.

L13. Using duckweeds for basic research in plant physiology: Exploring plant biological clock systems

Tokitaka Oyama

Department of Botany, Graduate School of Science, Kyoto University

Duckweeds are good materials for basic researches in plant physiology because of their morphological and developmental features. The tininess is an essential feature to use for various experiments in limited laboratory spaces. The two-dimensional structure of frond/colony gives an advantage in researches using imaging technologies. In the studies for the biological clock, relatively long and continuous observation is necessary to characterize rhythmic phenomena. Circadian rhythms of bioluminescence that are genetically introduced with a luciferase reporter are widely used for analyses of circadian systems because of its invasive, quantitative features. Bioluminescence reporter systems were applied to the detection of circadian rhythms of duckweed plants at a high spatio-temporal resolution, and we succeeded in monitoring circadian rhythms of individual cells in the plant as well as whole plants. The flat body floating on the medium surface and unchanging size/shape of a frond after completing the fast growth are a prominent character of duckweed to keep tracking bioluminescence at a single-cell level for a long term. Here advantages and also problematic issues in using duckweeds for basic physiology will be reported on the basis of the experience of our chronological studies.

L14. A new resource for cytogenetic studies in karyotype evolution of duckweeds

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The recently published genome sequence of *Spirodela polyrhiza* offers a suitable reference for investigations on genomic architecture of duckweeds, comprising 37 species with variable genome sizes (0.158 to 1.88 Gbp). By using consecutive multicolor fluorescence in situ hybridization (mcFISH) analyses, we align the 32 originally assembled pseudomolecules to the 20 small *S. polyrhiza* chromosome pairs. A Spirodela cytogenetic map containing 96 BAC markers with an average distance of 0.89 Mbp was constructed. Using a cocktail of 41 BACs in three colors, all chromosome pairs could be individualized simultaneously. Furthermore, seven ancestral blocks emerged from duplicated chromosome segments of 19 Spirodela chromosomes. Applications of this resource for studying on chromosome homoeology and karyotype evolution of duckweed species will be discussed.

The presentation is available at http://lemnapedia.org/wiki/A_new_resource_for_studies_in_karyotype_-genome-_evolution_of_duckweeds

L15. Spirodela polyrhiza post-transcriptional regulation responses to environmental and hormonal stimuli.

Paul Fourounjian[1], Jie Tang[2], Bahattin Tanyolac[1], Ying Zhang[2], Ying-Ying Zhang[2], Chris Wakim[1], Jiong Ma[2], Joachim Messing[1]
[1] Waksman Institute of Microbiology, Rutgers University, Piscataway
[2] School of Environment and Energy, Peking University Shenzhen Graduate School, Shenzhen

The Lemnaceae are the smallest, fastest growing, and most morphologically reduced angiosperm family, which present great potential in applications to wastewater treatment, biofuel, and animal feed production. The Spirodela polyrhiza genome was published last year, marking it as the representative genome of the family. We seek to characterize the post-transcriptional regulation of this plant in control conditions and in response to seven stimuli, including abiotic stress and hormone exposure. We are therefore analyzing small RNA libraries to identify conserved miRNA family members and novel miRNAs with miRPlant, and predicting their mRNA targets using psRNAtarget. We additionally prepared libraries of cleaved, uncapped mRNAs with the GMUCT 2.0 protocol. These libraries will allow us to align miRNAs with the targets they cleaved to validate miRNA function, and target gene predictions, using the sPARTA program. Finally, we are examining miRNAs that were differentially expressed, and targets that were differentially cleaved in each condition to characterize Spirodela

post-transcriptional responses. We hope to establish the first catalog of Spirodela miRNAs and targets, and investigate seven post-translational response patterns with this data to assist and guide future studies of this species and family.

L16. Making duckweed a crop plant: Accumulation of starch

Klaus-J Appenroth[1], K. Sowjanya Sree[2]

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In duckweeds, two of the physiological responses make them viable candidates for bioenergy feed-stocks: (i) The growth rates are the highest amongst flowering plants resulting in high amount of biomass, and (ii) high content of starch is accumulated in the whole vegetative plant body reaching up to 50 % of dry weight. Several methods are known meanwhile to induce accumulation of starch: lack of nutrients in the cultivation medium, salinity or heavy metal stress. In *Lemna minor*, clone 9441 we investigated the influence of phosphate and nitrate limitation, NaCl-induced stress and the effects of cobalt and other heavy metals on starch accumulation. However, the natural variation of starch accumulation in different duckweed species and clones has hardly been tapped at present. Effect of salt stress (NaCl) was investigated in 16 clones belonging to the species *Spirodela polyrhiza*, S. *intermedia*, *Landoltia punctata*, *Lemna aequinoctialis*, *L. disperma*, *L. gibba*, *L. minor*, *L. obscura*, *Wolffiella hyalina*, *W. lingulata*, *Wolffia arrhiza*, *W. columbiana*, and *W. globosa*. In some clones, starch accumulation is induced at NaCl concentrations which do not significantly inhibit their growth. This makes it possible to use a one-step procedure for the production of starch-rich biomass.

L17. How fast can duckweeds grow?

K. Sowjanya Sree[1] and Klaus-J Appenroth[2]

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Duckweeds are gaining the platform as crop plants because of one of their important features, high growth rate. *Wolffia* species being a candidate for use as food for human nutrition, we screened 33 clones of *Wolffia* belonging to all 11 species of this genus for their growth potential. A huge variation in growth rates was observed. Although in no other species, composition of the cultivation medium had a significant effect on the growth of *W. microscopica*. This recently rediscovered species is the fastest growing one in N-medium. The fast growing nature was correlated with the rate of photosynthesis and respiration. It is well documented that temperature plays a crucial role in growth of duckweeds. Interestingly, it was observed that in case of *W. microscopica*, higher temperature did not only affect its growth rate but also its morphology. At higher temperatures, the pseudoroot form which plays a key role in the identification of this species, turns into a flat form which lacks a visible pseudoroot. These flat forms are also the non- flowering stages of the fastest growing species.

L18. Different genetic patterns of two natural duckweed populations in China

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Duckweed is widely used in environmental biotechnology and has recently emerged as a potential feedstock for biofuels due to its high growth rate and starch content. Although duckweeds have been studied in the laboratory for many years, limited information is available on the biology of natural populations. Such data is essential for understanding the reproduction mechanisms in the natural population and should provide useful insights into the evolutionary processes at the population level. In this report, we present genetic characterization of two duckweed populations collected from Lake Tai and Lake Chao, China. Multilocus sequence typing (MLST) was employed for duckweed molecular characterization for the first time. Sharp differentiation on genetic stability was observed between the two duckweed populations. Moreover, phylogenetic analysis showed that MLST might be an effective tool for distinguishing closely related species. In a word, our work offers a basic framework for using MLST to characterize duckweed strains at the species level, and to study population genetics and evolution history of natural duckweed populations.

L19. Impact of the alien invasive species Lemna minuta on the congeneric native Lemna minor

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The use of duckweeds in wastewater treatment, biofuel production, fish and poultry farming and for many other purposes has considerably increased in the last few years. Practical applications of duckweeds commonly involve the use of species outside their natural, distribution area. If such species escape, they may become invasive and be a serious threat to local biodiversity. Considering the high growth rates of duckweeds and their ease of dispersion, it is important to know the factors that promote their spread.

In this study the competition between *Lemna minuta* and *Lemna minor* is tested under different environmental conditions.

The relative competitive abilities of these two species in Ireland were tested in the laboratory under different levels of nutrients, light and temperature. Laboratory results were interpreted in the contest of the distribution pattern of these two species in the wild. We found that *L. minuta* grew faster than *L. minor* at all nitrate concentrations tested. *L. minuta* also grew faster at high concentrations of phosphorus, high light

intensities and high temperatures. However, *L. minor* grew faster than the alien species *L. minuta* at concentrations of phosphorus lower than 500 μg/l (i.e. oligotrophic), under light regimes lower than 20 μmol · m^{2} · s^{I} and under temperature lower than of 15°C. In accordance, an outdoor experiment confirmed that *L. minor* has a higher growth rate than *L. minuta* in the colder months. The distribution of the two species in the natural environment did not, however, reveal that *L. minuta* was always dominant in nutrient-rich or well-illuminated habitats, indicating that additional parameters also play a role in determining competitive dominance.

L20. Accumulation of Selenium in Duckweed (Landoltia punctata) and Its Effects on Ultrastructure, Antioxidant Enzymes and the Chlorophyll a fluorescence OJIP transient

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Removal of selenium (Se) by duckweed is of interest in the control Se pollution. Also duckweed is useful as aquaculture feed. In this study, *Landoltia punctata* was grown over a 12 d period under concentrations of selenite (Na_2SeO_3) from 0 to 80 μmol L^{-1}. Lower Se concentrations of ≤ 20 μmol L^{-1} promoted *L. punctata* growth and inhibited lipid peroxidation (LPO). This anti-oxidative effect was caused by an increase in guaiacol peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) activity as well as amounts of photosynthetic pigments. Significant increases in growth rate and organic Se content in the duckweed were a consequence of Se enrichment. On the other hand, these changes were the opposite in *L. punctata* exposed to Se concentrations greater than 20 μmol L^{-1}. The changes of antioxidant enzymes and photosynthetic pigments were reflected in ultrastructure. The antioxidation and toxicity were correlated with Se bioaccumulation. It follows that the appropriate concentration of Se in the growth media for *L. punctata* should be less than 20 μmol L^{-1}. In summary, *L. punctata* has some tolerance to Se and can be used for treatment of Se-contaminated water. Duckweed with high Se content is useful as antioxidative food for aquaculture.

L21. The application research of duckweed on heavy metal remediation and wastewater treatment

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In the research of heavy metal tolerance strains screening, we screened out 49 duckweed strains from 350 strains under Pb^{2+}, Cr^{6+}, Cd^{2+}, Cu^{2+} or Zn^{2+} tolerance. After scientific secondary screening, we picked out the best strain, which growed 7 days in 1mg/L and 10mg/L Cd2+ medium, the duckweed strain could accumulate Cd2+ content 300~6150 mg/kg. Growed 10 days in 0.1mg/L and 0.2mg/L Cd2+ medium, this strain could absorb 48.21~66.29% Cd2+, and growth rate of dry weight were more than 3.03g/m2/d (surpassed CK). The results indicated that duckweed has good remediation results and application potential in all kinds of Cd2+ concentration.

We did the comparison study between duckweed and water hyacinth in two pilot-scale wastewater treatment systems for more than one year. The results indicated duckweed had the same TN recovery rate as water hyacinth and a slightly lower TP recovery rate even though its biomass production was half that of water hyacinth. Meanwhile, the higher content of crude protein, amino acids, starch, phosphorus, flavonoids and lower fiber content provided duckweed with more advantages in resource utilization. Additionally, microbial community indicated that more nitrifying bacteria and less nitrogen-fixing bacteria in rhizosphere of water hyacinth provided it with higher nitrogen removal efficiency, however, lower nitrogen recovery efficiency. In summary, duckweed has more application advantages than water hyacinth because it more effectively converted the wastewater nutrients into high-quality biomass. Meanwhile, this study tries to add plastic carriers into duckweed system to cover the shortage of duckweed root system and enhance the performance of microorganisms. The results indicated that adding biofilm carrier into a duckweed-based system did not affect significantly the duckweed growth, contents of protein, phosphorus and carbon, recovery rates of TN, TP and CO2 and the removal efficiency of TP. However, it improved significantly the removal efficiency of TN and $NH4^{+}$-N.The TN removal efficiency of the duckweed system with carrier closed to that of water hyacinth system. In addition, 454 pyrosequencing revealed a great difference of microbial communities between the different parts (surface water, bottom water, bottom sediment, carrier biofilm and rhizosphere of duckweed) in each system, and a significant similarity between the same parts of two systems.

L22. Duckweed powder as a replacement for fish meal in the feed used in Tilapia (GIFT) fry rearing

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Duckweed (DW) is a tiny aquatic plant that is rich with protein and could be produced at little cost and with little effort. It could be used as a protein providing ingredient to fish feed and could be reduced the cost of feed accordingly. The attempt of this study is identify the amount of DW powder that could be incorporated to basel fish feed of Tilapia fry rearing. The trial was carried out in 9 fiber glass tanks (500 L) outside. Tilapia advanced fry (Mean Weight =1.5906 ± 0.3054) were collected from Udawalawe NAQDA station. The used stocking density was 110 fry/tank. Fresh DW was collected from the pond in National Zoological garden in Colombo. The DW was separated and dried in sunlight and further dried in oven in 120°C and grind into powder. The feed (Basel Feed) for Tilapia was prepared (25%) using fishmeal as protein provider and the other 2 feed were prepared replacing 10% and 20% of this fishmeal through DW powder. Rearing period was lasted up to 70 days. The final Growth performance values of Basel feed, 10% and 20% DW included feed those are Final Mean Weight(3.3662 ±0.5137, 3.3918 ±0.1968 and 3.2596 ±0.0772 g), Mean Weight Gain (0.0254 ±0.0073, 0.0257 ±0.0028 and

0.0238 ±0.0011 g day-1), % Mean Specific Growth Rate (1.0593 ±0.2258, 1.0802 ±0.0819 and1.0245 ±0.0338), Mean Absolute Growth Rate (1.7756 ±0.5137, 1.8013 ±0.1968 and 1.6685 ±0.0772), % Mean Relative Growth Rate (111.63 ±32.29, 113.24 ±12.37 and 104.89 ±4.85), % Survival(78.13 ±12.62, 90.9 ±4.55;and 86.33 ±8.69) and Food Conversion Ratio (5.0318 ± 1.2131, 4.4860 ± 0.2712 and 4.6230 ± 0.2209) were not significantly different(p>0.05), respectively. In observation,DW powder included feed were attracted than the basel feed by the fish. Duckweed powder could be included to the used basel feed upto10% or 20% without affecting growth performance and survival of the fish Tilapia (GIFT) fry in rearing. Further research is needed to determine the maximum % that could be incorporated. Key words: Tilapia (GIFT), Duckweed (DW), Fry rearing.

L24. Duckweed as a platform for bioremediation and biomass production through interaction with C1-microbes

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C1-mirobes, e.g., methane- and methanol-utilizers, are ubiquitous habitants in the phyllosphere of many terrestrial plants.

We previously found that the aquatic plant surface is a niche for methane-utilizers. *In situ* activity of methane consumption by methanotrophic bacteria associated with different species of aquatic plants was ca. 370 fold higher than epiphytic methane consumption in submerged parts of emergent plants (Frontiers Microbiol., 2014). Subsequently, we started to explore the possibility to utilize duckweed as a platform for methane mitigation. Duckweed samples isolated from Lake Biwa showed high methane-consuming activity, indicating positive involvement of duckweed in methane mitigation in natural environment.

Duckweed is considered to be one of promising candidate for biomass production. Pink-pigmented methanol-utilizing bacteria are major phyllsphere microbes, and known to stimulate growth of various plants. However, molecular mechanism of this growth promotion is not well known. In this study, some *Methylobacterium* sp. was found to have a positive effect on duckweed biomass production, followed by high-throughput growth measurement system of duckweed (developed by Dr. Oyama and his colleagues). Since the duckweed growth is rapid, growth promotion could be detected within two weeks in micro-well titer plates. Using these systems, we started to follow chemical compounds, which affect duckweed growth and photosynthetic activity, by combination with *Methylobacterium* knock-out strains. These analyses gave several candidate compounds, which gave positive effects on duckweed growth.

These interactions of C1-microbes and duckweeds will be introduced.

L25. Yeast mannan promotes the growth of Lemna minor

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Yeast mannan is an outermost cell wall polysaccharide in *Saccharomyces cerevisiae*, that is composed of □-linked D-mannose units. Here we found that external addition of yeast mannan (Sigma, M7504) significantly increases the frond number, plant dry weight and chlorophyll content of *Lemna minor* after 10 days cultivation in sterile hydroponic culture condition. On the other hand, □-linked D-gluco-D-mannan and D-galacto-D-mannan of plants *Amorphophallus konjac* (Inagel mannan 100A) and *Ceratonia siliqua* (Sigma, #48230), respectively, did not show such effects on the growth of *L. minor*, suggesting that □-linked microbial mannan specifically has a potential for the plant growth promotion. In addition, the amount of yeast mannan in the culture was not significantly decreased after the plant growth. Although some polysaccharides are well known to function as elicitors to protect plants from pathogenic bacterial infection, direct plant growth promotion by microbial polysaccharide has never been reported.

We are now planning to prepare structural variants of mannan to identify the structure that is necessary for plant growth promotion. This research should contribute to understand novel plant-microbe symbiotic mechanisms and to develop ecological and effective plant cultivation.

L26. Estrogen degradation by utilizing the symbiosis of Rhodococcus zopfii Y 50158 and duckweed for developing eco-friendly water purification technology

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It has been a concern that estrogen, a steroidal female hormone, discharged into river and seawater would cause feminization of aquatic lives resulting to disrupt the ecological balance. Recent studies show that microorganisms in activated sludge of wastewater treatment plant (WWTP) have ability to degrade estrogen compounds. However, there are reports that removal of estrogens is still incomplete in WWTP and estrogens in direct effluent from ranches remain untreated. Therefore, establishment of an effective and eco-friendly remediation technology eliminating estrogen is required. Rhizoremediation is a type of bioremediation technology that utilizes activation and stabilization of useful bacteria in the rhizosphere of host plants. We had shown that a symbiotic system of a rhizobacterium *Acinetobacter calcoaceticus* P23 and *Lemna aoukikusa* continuously degraded phenol for a week and also enhanced the plant growth. We believe that rhizoremediation is one of the solutions for above requirement.

Rhodococcus zopfii Y 50158 (Yakult Central Research Institute) was previously isolated from activated sludge as an estrogen degrading bacterium strain. Strain Y 50158 has high estrogen degrading activity enough to degrade 100 ppm of estrogen (E2: -estradiol) in 24 h. In order to expand the application range of duckweed rhizoremediation system, we were motivated to examine the ability of the combination of a foreign non-rhizospheric bacterium strain Y 50158 and *Lemna minor*. Strain Y 50158 was previously adhered to sterile *L. minor* and their estrogen degrading ability and sustainability were examined. They were successfully shown to degrade estrogens but *L. minor* gradually got out of condition, showing limited sustainability.

We are now planning to co-adhere plant growth-promoting bacteria and *R. zopfii* strain Y 50158 to *L. minor* in order to maintain the healthy growth of the duckweed.

L27. The Duckweed a Valuable Bioenergy Plant for Biofuel Production and Waste Water Treatment

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Duckweed has been considered as a valuable feedstock for bioethanol production due to its high biomass and starch production. In this presentation, I will show our research progress in duckweed resource collect in China, special characteristics stain selection, influence factors in duckweed starch accumulation, transcriptomics investigation of duckweed starch accumulation, space-saving duckweed cultivation model development and large-scale duckweed cultivation system development using waster water from cow cultivation. For duckweed resource and strain selection, 311 geographic isolated duckweed strains were collected from 22 provinces in China, after large-scale screening, *Lemna aequinoctialis* 6000 from Hunan province was obtained with the highest starch accumulation in SH medium. As to influence factor in duckweed starch accumulation, we had investigated the starch content variation in one day, and the effect of different culture medium and salt concentration on *L. aequinoctialis* 6000 starch accumulation. To further investigate the effects of light conditions on duckweed biomass and starch production, *L. aequinoctialis* 6000 was cultivated at different photoperiods (12:12, 16:8 and 24:0 h) and light intensities (20, 50, 80, 110, 200 and 400 μmol m^{-2} s^{-1}). Moreover, comparative analysis of duckweed cultivation with sewage water and SH media for production of fuel ethanol was done in our group. Further, a highly branched arabinogalactan named DAG1 (Mw~4.0×10^4 Da) was purified from *L. aequinoctialis* 6000. Nitrogen-starvation could induce starch accumulation in duckweed. To further investigate the starch accumulation in *L. aequinoctialis* 6000, transcriptome was analyzed after cultivated in nitrogen-starvation for 0, 3 and 7 days. To promote the duckweed commercialization, space-saving cultivation model was designed. Further, we established 1500 m^2 duckweed large-scale multilayer cultivation system using waste water from cow cultivation. The duckweed was cultivated in photovoltaic green house to keep the system working in the whole year. Moreover, duckweed starch automatic isolation system was developed in our group.

L28. Rolling Up Our Sleeves, Creating the Duckweed Industry from the Water Up

Tamra Lynn Fakhoorian
International Lemna Association

Outline of early-stage industrial development of duckweed to present day, address current production and processing challenges, offer specific opportunities for researcher engagement, and outline conceptual business models based on real-world trends in climate and water challenges, human population growth, fishmeal depletion, and GMO attitude shifts.

The full presentation is available at http://lemnapedia.org/wiki/Rolling_Up_Our_Sleeves,_Creating_the_Duckweed_Industry_from_the_Water_Up

Posters abstracts

P1. Co-adhesion to Lemna minor of rapidly colonizing bacteria and Acinetobacter

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Background and Objectives: In our laboratory, environmental purification systems utilizing symbiotic relationship between bacteria and *Lemna minor* have been developed. Yamaga isolated *Acinetobacter calcoaceticus* P23 that rapidly colonized on the surface of *Lemna* and continuously degraded phenol for a week in the culture medium. At the same time, P23 increased the frond number of *L. minor* by 1.7 times more than *L. minor* only (Yamaga *et al.*, 2010). However, stability of P23 on *L. minor* was not so high as expected in a wastewater effluent. In this study, we aimed to construct multi-species bacterial consortium that stabilizes P23 colonization on *L. minor*. We previously noticed that some bacteria in pond water had ability to enhance the adhesion of P23 to *L. minor*.

Materials and Methods: Rapidly colonizing bacterial candidates to sterilized *L. minor* were isolated from the pond water after 4 h adhesion and gentle surface rinse. In order to verify their rapidly colonizing ability, sterilized *L. minor* was grown for 4 h in each bacterial suspension (OD_{600}=0.3) and CFU (colony forming unit) were separately measured in the root and frond part. Finally, the effect on the stability of P23 was compared by co-adhesion of P23 and each isolated bacterium.

Results and Discussion: P23 was found to adhere to frond much more than root part. Then, ten bacterial strains that dominantly attached to root were selected. When sterilized *L. minor* was cultivated in mixed suspension of each bacterium and P23, two among ten bacteria were found to improve early adhesion of P23 to *L. minor*. Both of these two strains were identified as genus *Pseudomonas.* However, stability of P23 colonization and its plant growth-promoting activity were not improved by these strains when compared to only P23. Our results suggest that there exists a highly sophisticated microbial population balance on the surface of duckweed.

P2. Effect of aquatic plant growth-promoting bacteria on the growth of rice

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[Background and Objectives]: In natural environments, living organisms influence each other directly or indirectly and form complicated ecosystems. There are a number of reports on the symbiosis between plants and their associated microorganisms. Bacteria that promote the growth of plants are called PGPB, plant growth-promoting bacteria. *Acinetobacter calcoaceticus* P23, isolated from duckweed, *Lemna aoukikusa*, at the Hokkaido University Botanical Garden, is a PGPB for duckweed. It was also shown that P23 is capable of increasing the chlorophyll content of not only a monocot *Lemna minor* but a dicot *Lactuca sativa* (Suzuki *et al.* 2014). On the other hand, PAE2, PAE9, PAR7, and PAR21 are PGPB of Poaceae plant reed, *Phragmites australis*. We were thus prompted to examine whether these bacterial strains have growth-promoting effects on Poaceae grain rice, *Oriza sativa* cv. Nipponbare.

[Methods and Results]: After dormancy breaking followed by germination, young seedlings were pre-cultured for a week in 1/5x Kimura B liquid medium. Seedlings were first grown for a week in the medium containing bacteria and were further grown for another week in a new medium without bacteria. It was found that P23, PAR7 and PAR21 were capable of increasing the biomass and chlorophyll content by 20 to 50%. Increase in the number of tillers was also observed for PAR21. Colonization of the bacteria on the root was verified by fluorescent microscopy using BacLight staining kit.

P3. Effects of environmental bacterial community on growth of duckweeds

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Growth of duckweed are very influenced by several environmental factors, including temperature, nutrient concentration and light intensity. In addition to these factors, environmental bacteria also affect the growth of duckweed. Effects of bacteria on the plant growth have been studied in terrestrial higher plants. Plant growth-promoting bacteria (PGPB) provide some benefits to plant. On the other hand, some bacteria have negative effects on plant. However, the interactions between environmental bacteria and duckweed remain unclear. In this study, we studied the effects of environmental bacteria on duckweeds.

We collected environmental bacterial communities from several points. We cultured duckweeds (*Lemna minor*, *Spirodela polyrhiza*, *Wolffia arrhiza* and *Wolffia globose*) with and without bacterial community in Hoagland solution. We monitored growth, chlorophyll content and photosynthesis of duckweeds.

P4. Characterization of microbial consortium and methane oxidation in the methanotroph-duckweed symbiotic system

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Aquatic environments such as wetland, lake and paddy field are main methane sources. Recently, we found that submerged parts of aquatic plants were colonized by methane-utilizing bacteria (methanotrophs), with high methane oxidation potentials. This indicates that methanotrophs living on aquatic plants can act as a biofilter to reduce methane emitted to the atmosphere. In this study, we obtained methane-utilizing microbial consortium including methanotrophs from duckweeds in Lake Biwa, Japan, and analyzed methane oxidation in the methanotroph-duckweed co-culture system.

Metagenomic analysis of duckweeds which had methane consumption activity revealed that many kinds of methanotrophs colonized on duckweeds and that type I methanotrophs, such as *Methylobacte*r sp. and *Methylomonas* sp., predominated over type II methanotrophs, such as *Methylocystis* sp. and *Methylosinus* sp., on duckweeds. We isolated one strain of methanotroph, *Methylomonas* sp. BLU1 from the duckweed sample and used for methane consumption analysis with sterilized duckweeds, *Spirodela polyrhiza*. Strain BLU1 consumed methane more quickly in the presence of duckweeds than without duckweeds, indicated that methane consumption of methanotrophs was stimulated by duckweeds.

P5. Effects of external addition of plant hormones and some minerals on the growth of duckweed Lemna minor

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Effect of plant hormones, such as auxin, ethylene, gibberellin, and salicylic acid, have been well-studied in terrestrial plants, however their effects on aquatic plants are yet unknown. We were thus prompted to know the effects of these compounds on the Duckweed, *Lemna minor* growth. The growth of *Lemna minor* was tested for 10 days in Hoagland medium supplemented with each plant growth-promoting compound at 0-100 μM concentrations. Among the seven compounds tested, Gibberellin exhibited no apparent effect in all aspects of plant growth. Salicylic acid did not increase the plant growth but increased the chlorophylls content at concentrations higher than 10 μM. A precursor of ethylene production, 1-aminocyclopropanecarboxylate, inhibited the growth of *Lemna minor* at all concentrations. An inhibitor of ethylene production, aminoethoxyvinylglycine, exhibited strong phytotoxicity. These results demonstrate that the action of phytohormones to the growth of duckweed, *Lemna minor*, is largely different from that of terrestrial plants.

On the other hand, indole 3-acetic acid (auxin) clearly increased the fronds number and body weight at 5 μM or less of the concentration range. Fe-EDTA also significantly increased the growth of plant at 1 μM and decreased the growth at higher concentrations, 50 to 100 μM in 12 μM $FeSO_4$ containing medium condition. Phosphate was required for the healthy growth at least 30 μM.

P6. Effects of external organic compounds on growth and turion formation of rootless duckweed Wolffia arrhiza

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Rootless duckweed *Wolffia arrhiza* is applicable for removal of nutrients from wastewater because it grows quickly and absorbs large amounts of nutrients. In addition, vegetative fronds and turions of *W. arrhiza* are valuable as a protein resource and a starch resource, respectively. Therefore, a cultivation system of *W. arrhiza* is a promising co-beneficial system for water purification and resource production. This study examined effects of external organic compounds on the growth and turion formation of *W. arrhiza*. The organic compounds were investigated as possible factors to improve starch productivity. Among the tested organic compounds, certain saccharides such as sucrose, fructose, maltose and cellobiose enhanced the growth and turion formation of *W. arrhiza*. Although other organic compounds did not significantly enhance its growth, glucose, citrate, pyruvate, and succinate slightly induced the turion formation of *W. arrhiza*. The addition of both sucrose and inorganic nitrogen promoted effectively the starch production by *W. arrhiza*. Results show that efficient systems which contribute to nutrient removal and starch production can be established using *W. arrhiza* by addition of proper organic compounds.

P7. Developing a manipulation system of partial illumination to the microarea of duckweed plants for the detection of intercellular signaling on cellular circadian clocks

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The circadian clock system is basically cell-autonomous in plants and circadian clocks of individual cells are presumably influenced by other cells. Thus, the circadian system of a tissue and plant body is likely to be represented as the union of individual cellular clocks. In order to understand the pattern of intercellular communication among cellular circadian clocks in plants, we utilized a single-cell bioluminescence monitoring system for duckweed plants. Bioluminescence monitoring systems using circadian-regulated luciferase reporters have been applied to the analysis of circadian clock system of plants. Using a transient expression system with a particle bombardment method, circadian rhythms of duckweed plants were conveniently observed (Miwa et al. 2006). *Lemna gibba* is a duckweed plant that has been used for the study of circadian rhythms and photoperiodism. In order to observe the light signal transmission between cells in the plant body,

we developed a manipulation system of illumination on a microarea in duckweed plants for long-term experiments of circadian rhythms. We combined this device with the bioluminescence reporter imaging technology. We used a 100-micro meter optical fiber and made a device to fix a fiber end to a position in a frond. LED-light guided by the optical fiber illuminated a microarea on the frond during monitoring of cellular bioluminescence for circadian rhythmicity. Using this apparatus, we succeeded in measuring bioluminescence of individual cells under partly-illuminated conditions for 10 days. Under the monitoring conditions, synchronized cells to light/dark cycles around the light-illuminated area and free-running cells were simultaneously observed in the same frond. An ablation experiment that partially divided the frond was conducted. Single-cell bioluminescence in both parts of the frond was observable over 10 days. We will present these "classical" physiological experiments using duckweed in combination with advanced technologies.

P8. Evaluation of growth of Lemna gibba under various photoperiod conditions

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Most organisms anticipate daily changes on a rotating planet using the endogenous circadian clock that is synchronized with the external environmental cycle. Plants use solar energy for photosynthesis, accumulate sugars/carbohydrates in the daytime and consume them in the nighttime. The regulation of daily changes in metabolisms involves the circadian clock and it is crucial that the phase (time) of the clock is synchronized with environmental day-night cycles. In studies using *Arabidopsis thaliana*, it was reported that matching the periods of between the internal circadian clock and external light-dark cycles increases productivity. This is termed "circadian resonance" between circadian rhythm and environment. However, it is little known whether circadian resonance occurs similarly in other plant species. We tried to analyze circadian resonance of duckweeds. Previously, we developed a technique to observe the duckweed circadian rhythm using circadian-expressed bioluminescent reporters and characterized period lengths of circadian clocks of duckweeds under constant conditions (Muranaka et al., *Plant Biol.*, 2014). Then, we evaluated growth rates of *Lemna gibba* under various light-dark cycles and effects of sucrose in the culture medium. Here, we present the dependency of photoperiods on the growth and involvement of sucrose in this process. Recently, duckweeds attract industrial attention, due to their fast-growing ability and high contents of proteins and carbohydrates. The biomass of duckweeds can be utilized as biofuels and feed for livestock. Thus, the outcome from the studies on effects of light conditions on duckweeds' growth will be useful in the improvement of productivity.

P9. Diversity of circadian rhythms and photoperiodic responses in duckweed

Tomoaki Muranaka, Tokitaka Oyama

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Duckweeds have been used in many physiological experiments because of their unique characteristics such as tiny plant bodies, rapid growth rates, vegetative reproduction and strictly controllable environments under aseptic conditions. In addition, the diversity of the photoperiodic response in flowering made some Lemna species model plants of the study on photoperiodism. In plants, the photoperiodic responses are based on the day-length measurement by circadian clock. We therefore developed the bioluminescent reporter system for analyzing the diversity of the circadian system in duckweeds. In this system, the luciferase gene driven by the promoter of a circadian-controlled gene was introduced into duckweeds by a particle bombardment technique. The luminescent rhythms were monitored by an automated bioluminescent monitoring system with a photomultiplier tube. We compared the traits of circadian rhythms of five duckweed species across four genera (*Lemna gibba, L. aequinoctialis, Landoltia punctata, Spirodela polyrhiza, Wollfia columbiana*). Under 12-h light/12-h dark cycles, all tested duckweeds showed clear diel rhythms. This suggests that the mechanisms of scheduling biological timing under day–night cycles are conserved in duckweeds. Under constant conditions, circadian rhythms showed diversity in period lengths and sustainability, suggesting that circadian clock mechanisms are somewhat diversified among duckweeds. We then analyzed the intra-species diversity of circadian traits and photoperiodic flowering using Japanese *L. aequinoctialis*, the short day plant showing the latitudinal cline in the critical day-length for flowering. We found a correlation between circadian period and critical day-length. This suggests that the diversity of circadian rhythms might be caused by the modulation of critical day-length for local adaptation.

P10. Improvement of fast and efficient Agrobacterium mediated stable transformation methods for Lemna species

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The duckweeds are fast growing floating aquatic monocots, the smallest and morphologically simplest of flowering plants. Before *Arabidopsis* becomes a model plants for basic plant research, since the duckweeds are small and asexually propagate under aseptic conditions, they are used as the ideal system for plant biological research. For example, much knowledge about circadian clock and photoperiodic responses comes from physiological researches conducted on the *Lemna* species. Recently, whole-genome sequence of *Spirodela polyrhiza* was reported and genome projects of some *Lemna* species are also currently in progress now. Therefore it is expected that not only basic plants researches as well as commercial applications (biofuel, pharmaceutical products, phytoremediation *etc.*) will accelerate using duckweeds. For further acceleration of duckweeds researches, in addition to genome resources, establishing genetic engineering tools are also required. Among them stable transformation methods in duckweeds are long awaited techniques. We have already established an efficient transient transformation system in the duckweeds by a particle bombardment technique and been analyzing circadian clock systems at the single-cell levels by bioluminescence reporters. Here we show recent progress to establish the fast callus induction, fronds regeneration and efficient agrobacterium-mediated transformation techniques in several *Lemna* species.

Duckweed anthem

by Paul Fourounjian

Gathered here in Kyoto
We share knowledge of duckweeds
We learn from the professors
Appenroth and Toki
Of new ways to fix old problems
And the wonders of biology
They're the smallest and the fastest
A model of simplicity
They teach us all a lesson
On the power of neoteny
--

(Chorus)
Not even Landolt knew
What these plants could do
So he studied them through
And through
Now we take his lead
And carry on
And test this question too
And the work goes on
As the planet warms
Until everyone sees
What these plants can do
And how much we need
The power of duckweeds
–

Luckily, The great plant thrives
In our own waste streams
Dividing as, they live their lives
To keep our waters clean
Then just like The Giving Tree
They offer up their bodies
So small and easy to harvest
For our food and energy.
We thank you once again duckweeds
For your generosity
–

(Chorus)
Personal verse *(everyone can make their own)*
The genome map was published
For all of us to see
Yet we still improve upon
our cartography
So I paint the tiny fragments now
And their silent activity

(Chorus, final, slowly)
Each day we work to understand
The power of duckweeds.

You can hear the anthem in http://lemnapedia.org/wiki/The_Duckweed_Anthem

References that might not be recognized by everyone:

- Kyoto 2015, 3rd International Duckweed conference on research and applications.
- Appenroth, Toki, Landolt: Distinguished duckweed professors.
- Neoteny: The retention of juvenile characteristics in an adult organism.
- Giving Tree: A poem of a tree, that takes care of a boy, until he's a man. Then she tells him to cut her down to build a house and boat for his family. When she's a stump she still offers herself as a seat.

There are certain Rugby songs where people can take turns putting in their own verse, before returning to the chorus that everyone knows. I see it as a good system to encourage people to personalize and expand the song, a few lines at a time.

While I sing this as a Rugby song, I hope that other researchers can modify the lyrics, and use whatever melodies they like to personalize this song to their tastes, so it can grow and evolve like any good idea or organism.

Paul F.

Invitation to the next ICDRA 2017 in India

Dear colleagues of the duckweed community,

My heartfelt thanks to all the participants of the “3rd ICDRA” in Kyoto for giving me the opportunity to organize, the “4th ICDRA” in India in 2017.

India is a paradise for duckweeds. Although eleven different species belonging to Lemnaceae family occur naturally in different parts of India, four of them are very common: *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna aequinoctialis* and *Wolffia globosa*. Rare species like *W. microscopica* are even endemic to the Indian sub-continent.

I cordially invite one and all of the duckweed colleagues and enthusiasts to actively participate in the 4th ICDRA in India and I look forward to welcome you all to a land rich in biological and cultural diversity.

We would come up with more precise information on the meeting by the end of this year in the coming issues of the ISCDRA newsletter.

WELCOME TO INDIA!!!

Namaste!

With best wishes,

Sowjanya

Dr. K. Sowjanya Sree

Chair, 4th ICDRA

India