ISCDRA

International Steering Committee on Duckweed Research and Applications

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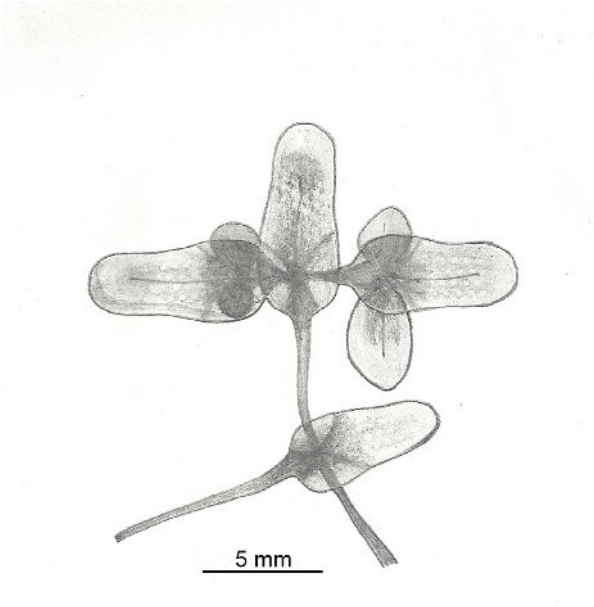
More information about the ISCDRA and previous issues are available at
http://lemnapedia.org/wiki/ISCDRA

Science meets Art: *Lemna trisulca L.*



Lemna trisulca L. is widely distributed in nature especially in the cooler climatic zones. These plants store starch before the arrival of unfavorable climatic conditions and sink deeper in the waters. Stipes (tissue connections between the mother and the daughter fronds) are highly stable and as a consequence not only small colonies can be observed as in most other species but very long chains of several generations of fronds are formed. This feature perhaps makes it difficult to handle *L. trisulca* in the lab restraining its use in research (except for study of blue-light induced chloroplast movement). Drawing: Dr. K. Sowjanya Sree, Amity University UP, India (kssree@amity.edu; ksowsree@gmail.com).

Letter from the Editor

Dear Duckweed friends,

You are receiving the volume 3, issue 3 of the Newsletter immediately before the **3rd International Conference on Duckweed Research and Applications in Kyoto, Japan from 3rd to 6th July, 2015**. We are looking forward seeing many duckweed friends there and to learn about the newest progress in research and applications. The programme from the organizers is very promising. This is the last issue published by the first International Steering Committee on Duckweed Research and Applications. I would like to thank especially Tamra Fakhoorian, International Lemna Association, USA and Eduardo Mercovich, MamaGrande, Argentina who managed the editorial work with me. The next issue will be edited by the already newly-elected second Steering Committee. Please, find the results of the election in this issue.

In this issue, you will also find a report from the company "GreenOnyx", Israel. We are proud to feature the first of what we expect will be many white papers by new companies engaged in duckweed-related products and services. The presentation of another start-up company in the next issue is already in preparation. "Applications" is reaching the point of economic significance which is urgently required in our field.

John Cross, known as the editor of the famous website "The Charms of Duckweed" (http://www.mobot.org/jwcross/duckweed/duckweed.htm), writes about rise and fall of a biotechnological duckweed company from the US with lots of highly interesting facts.

As started in the previous issue, we continue to report about "Useful Methods" and "Experiments for Students". We invite again other researchers and teachers from school, colleges or universities to contribute in the next issues about these topics in order to increase the interests in duckweed all over the world.

As always, "From the Data Base" forms again a serious part of our Newsletter. We want to inform all interested people about the present state of art in the scientific duckweed literature.

We hope you will find also this issue interesting and entertaining.

On behalf of the ISCDRA

Klaus-J. Appenroth

Election for the 2nd ISCDRA Members

The original 2 years term of the 1st ISCDRA is ending, and the next one will commence after the Kyoto meeting.

In order to define the new 5 members, we followed a simple procedure: after a call for Candidates (open to everyone who attended any ICDRA), the Candidates where contacted to verify their approval, the list was published and -again- everyone who attended the ICDRA was invited to vote up to 5 persons.

These are the results.

Votes	Area	Institution	Candidate	Country
29	Research	University of Jena	Dr. Klaus-J. Appenroth	Germany
27	Research	Rutgers University	Prof. Dr. Eric Lam	USA
26	Application	International Lemna Association	Tamra Fakhoorian	USA
19	Application	MamaGrande	Eduardo Mercovich	Argentina
18	Research	Virginia State University	Dr. Louis Landesman	USA

The newly-elected ISCDRA will have a meeting immediately after the Kyoto Conference, to define it's first steps.

The Rise and Fall of a Duckweed Biotechnology Firm: What can it tell us?

By John W. Cross[1,2]

John W. Cross, the famous author of "The Charms of Duckweed"

Introduction

Duckweeds are charming. By that, I mean these smallest flowering plants have unique characteristics that draw our scientific curiosity. But beyond intellectual fascination, several attributes provoke serious interest in the potential of duckweeds as a practical platform for biotechnologies. These include rapid growth, high protein and low fiber content, ease of axenic culture in simple media, and rapid uptake and metabolism of nitrogen and phosphate compounds.[3],[4],[5] Janet Slovin[6],[7],[8] at the USDA ARS and Anne-Marie Stomp[9] at North Carolina State University (NCSU) recognized this potential and demonstrated the ease of genetic manipulation of duckweeds in the laboratory using the techniques of Mendelian genetics, tissue culture, and Agrobacterium-based genetic transformation. Their results encouraged these pioneers and others to envision bioengineered duckweeds as a platform for manufacturing valuable biopharmaceutics or for the efficient remediation of wastewater. A key driver for Stomp's effort was the potential competitive value of an inexpensive production system for manufacturing biopharmaceutics. In 2005 Stomp estimated the market for pharmaceutical proteins is worth about $10 billion yearly and it clearly has

1 Alexandria, Virginia USA.

2 The author wishes to thank Lynn Dickey, formerly of Biolex and Synthon, for critical reading of the manuscript and making suggestions for improvement.

3 Landolt, E. 1986. The family of Lemnaceae– A monographic study, vol. 1. Veröff. Geobot. Inst. E. T. H. Stiftung Rübel Zürich 71.

4 Landolt, E. and R. Kandeler. 1987. The family of Lemnaceae– A monographic study, vol. 2. Veröff. Geobot. Inst. E. T. H. Stiftung Rübel Zürich 95.

5 Cross, J.W. Duckweed as a Primary Feedstock for Aquaculture. (1994) URL: http://www.mobot.org/jwcross/duckweed/Fish.htm

6 Slovin JP, Tobin EM. Synthesis and turnover of the light-harvesting chlorophyll a/b-protein in *Lemna gibba* grown with intermittent red light: possible translational control. Planta. 1982 Sep;154(5):465-72.

7 Slovin JP, Cohen JD. Levels of Indole-3-Acetic Acid in *Lemna gibba* G-3 and in a Large Lemna Mutant Regenerated from Tissue Culture. Plant Physiol. 1988Feb;86(2):522-6.

8 Tam YY, Slovin JP, Cohen JD. Selection and Characterization of [alpha]-Methyltryptophan-Resistant Lines of *Lemna gibba* Showing a Rapid Rate ofIndole-3-Acetic Acid Turnover. Plant Physiol. 1995 Jan;107(1):77-85.

9 Stomp AM. "The duckweeds: A valuable plant for biomanufacturing." Biotechnol Ann. Rev. 2005;11:69-99.

grown since then, with the US biotechnology drug market growing at an annual rate of 9.7% between 2010 and 2012.[10] Conventional biopharmaceutical manufacturing in mammalian cell cultures at that time were low-yield, extremely expensive and entailed complex systems for avoiding contamination with viruses and prions.[11],[12]

NCSU

This promise encouraged NCSU to assist Stomp in developing patentable technologies that could be spun off as a biotechnology firm. As the basis for this effort, Stomp was able to acquire the duckweed culture collection of Elias Landolt at the ETH in Switzerland under an NSF Grant. She also obtained an EPA Grant to investigate the genetic manipulation of duckweed for applications in wastewater treatment,[13] but nothing of practical note appears to have been done to follow up on that application. The EPA Grant did allow Stomp to pursue work on duckweed tissue culture[14],[15] that was the basis for her early genetic transformation experiments. Stomp was granted US Patents for this work (see table US Patents Attributable to Spinoffs from NC State Duckweed Research). In 1997 Stomp spun off Biolex Therapeutics to exploit applications of her research.

Biolex

Beginning in 2001, Ann-Marie Stomp and her team began filing patents assigned directly to Biolex. About that time Biolex began patenting technologies for automating growth and genetic manipulations with duckweeds.[16, 17,18,19] Ann-Marie Stomp left Biolex and returned to NCSU in 2002 to resume her university position. Biolex continued operations for a total of 12 years and over this time raised $190 million from investors.

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10 Hadjivasiliou, A. Pharmaceutical & Biotech Sales Analysis by Country: Top Drugs, Top Regions. Evaluate Pharma, May 2014. URL: http://www.evaluategroup.com/Public/Reports/EvaluatePharma-World-Preview-2014.aspx

11 Zhang J. Mammalian Cell Culture for Biopharmaceutical Production. In book: Manual of Industrial Microbiology and Biotechnology, Edition: 3rd ed., 2010, Chapter: 12, Publisher: ASM Press, Washington, DC., pp.157 - 178.

12 Kelley B. Industrialization of mAb production technology: the bioprocessing industry at a crossroads. MAbs. 2009 Sep-Oct;1(5):443-52. Epub 2009 Sep 16. Review. PubMed PMID: 20065641; PubMed Central PMCID: PMC2759494.

13 Stomp, Ann-Marie. Final Report: Genetic Improvement of Duckweed (Lemma Gibba) Wastewater Treatment. EPA Grant Number: R823570, (June 26, 1995 through June 25, 1998). URL: http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/475/report/F

14 Moon HK, Stomp AM. Effects of medium components and light on callus induction, growth, and frond regeneration in *Lemna gibba* (Duckweed). In Vitro Cellular & Developmental Biology-Plant 1997;33(1):20-25.

15 Moon HK, Stomp AM. Effects of media components and phytohormones on in vitro frond proliferation of *Lemna gibba* G3 and 24 additional *Lemna gibba* strains. Plant Research 1998;1(2):98-104.

16 Everett, K. Led array for illuminating cell well plates and automated rack system for handling the same United States Patent 6,680,200, January 20, 2004.

17 Everett, K. LED array for illuminating cell well plates and automated rack system for handling the same. United States Patent 7,160,717, January 9, 2007

18 Branson , RE et al. Bioreactor for growing biological materials supported on a liquid surface United States Patent 7,176,024, February 13, 2007

19 Everett, K. Plate and method for high throughput screening. United States Patent 7,326,385, February 5, 2008

Biolex took advantage of North Carolina state benefits in 2001 by renovating an abandoned textile mill in the small town of Pittsboro, not far from NC State. The company then expanded rapidly, acquiring Epicyte Pharmaceutical in 2004[20] and the French biotech company LemnaGene in July 2005[21] and again expanding their biomanufacturing facilities to a total of around 13,000ft^2 with GMP compliance by 4Q 2005.[22] Investor-funded R&D at Biolex enabled development of a complete system for manufacturing recombinant pharmaceutical-quality proteins in *Lemna minor*. Biolex dubbed their manufacturing technology the LEX System, and it demonstrated a number of advantages for manufacturing recombinant proteins. For example, it is suitable for making high-demand monoclonal antibodies and other glycosylated therapeutic proteins. Using RNA interference (RNAi) technology, Biolex researchers were able to engineer expression in *Lemna* of mAbs with a glycosylation pattern more like human antibodies.[23] The system was also capable of making virus-like particles suitable as vaccine platforms.[24,25]

However, it all came to an end when, in 2012, the company announced it would liquidate.[26] At its peak, Biolex had more than 100 employees, but planned to raise additional funds through an IPO in 2008 but were frustrated by the eruption of the financial crisis that year. As a result the success of the company depended critically on the company bringing to market at least one highly-profitable product in the absence of additional investor funding. The company was unable to accomplish this by the time funding ran out in 2012.

Business Decisions

Although the LEXSystem had been demonstrated to be capable of being used to manufacture a range of potentially lucrative pharmaceutical proteins, Biolex decided to bet the company on brining a single product to market. Alfa interferon is a well-studied protein that had been used to treat infectious diseases, including hepatitis B, hepatitis C and certain cancers. Biolex chose to develop a recombinant alpha interferon made using LEX System together with a proprietary controlled-release formulation from a Netherlands company, OctoPlus. The Biolex plan was to develop this product, called Locteron,

20 Tech Journal. Biolex acquires Epicyte Pharmaceutical. May 6th, 2004. URL: http://www.techjournal.org/2004/05/biolex-acquires-epicyte-pharmaceutical/

21 PR Newswire Biolex Therapeutics Acquires LemnaGene S.A. July 28, 2005. URL: http://www.prnewswire.com/news-releases/biolex-therapeutics-acquires-lemnagene-sa-54821487.html

22 Biolex Therapeutics Facility Expansion, Pittsboro, North Carolina, United States of America.

23 Cox KM, Sterling JD, Regan JT, Gasdaska JR, Frantz KK, Peele CG, Black A, Passmore D, Moldovan-Loomis C, Srinivasan M, Cuison S, Cardarelli PM, Dickey LF. Glycan optimization of a human monoclonal antibody in the aquatic plant *Lemna minor*. Nat Biotechnol. 2006 Dec;24(12):1591-7. Epub 2006 Nov 26. PubMed PMID:17128273.

24 Nguyen LV, Cox KM, Ke JS, Peele CG, Dickey LF. Genetic engineering of a Lemna isoleucine auxotroph. Transgenic Res. 2012 Oct;21(5):1071-83. doi: 10.1007/s11248-012-9594-2. Epub 2012 Feb 5. PubMed PMID: 22311339.

25 Bertran K, Thomas C, Guo X, Bublot M, Pritchard N, Regan JT, Cox KM, Gasdaska JR, Dickey LF, Kapczynski DR, Swayne DE. Expression of H5 hemaglutinin vaccine antigen in common duckweed (*Lemna minor*) protects against H5N1 high pathogenicity avian influenza virus challenge in immunized chickens. Vaccine. 2015 Jun 8. pii: S0264-410X(15)00749-5. doi: 10.1016/j.vaccine.2015.05.076. [Epub ahead of print] PubMed PMID: 26067184.

26 Carroll, John. Biolex to liquidate after burning $190M on hep C drug. FierceBiotech. July 9, 2012 URL: http://www.fiercebiotech.com/story/biolex-liquidate-after-burning-190m-hep-c-drug/2012-07-09

through mid-stage clinical trials and then to sign on a partner for the more expensive Phase III pivotal trials. Phase I clinical trials were completed in 2005[27] and Phase II studies were completed by November 2011.[28] However, by early 2012 Biolex's funds were rapidly dwindling and new financing was unobtainable. Although alpha interferon for Hepatitis C had been one of the most lucrative indications in biopharmaceutics, a new generation of medicines arose that cured the disease permanently without the need for interferon. This therapeutic development permanently eliminated a major market for Locteron.

Purchase by Synthon

After Biolex failed to raise new investor funding, its assets were put up for sale. Synthon, a Netherlands-based biotechnology company, who had previously been a Biolex collaborator, took an interest. Synthon was already active developing new biotherapeutic technologies, and had an existing facility in North Carolina's Research Triangle Park (RTP). Synthon bought the Lemna platform technology, the LEX System, and transferred six of the Biolex scientists and their research materials to RTP. Synthon redubbed the platform technology the Synlex System. Synthon began developing new molecular tools to take advantage of their investment and started applying for new patents.[29] However, Synthon's interests in their Synlex System declined over the first two years of ownership, and eventually the company abandoned the investment and laid-off the remaining duckweed scientists. The exact reasons Synthon lost interest have not been made public, but it seems likely that they had not identified a specific pharmaceutical product that needed to be made in duckweed. Industrial managers must always balance the risks of new technologies versus their advantages, and the Synlex system may not have made the quantitative cut. At some time in the future Synlex might have identified such a product, but no for-profit company can afford to keep scientists on the payroll waiting to manufacture an ideal product for their technology if one is not already at hand.

Afterwards

This article is a case study to show how uncontrollable risk factors can determine the success or failure of a duckweed-based technology project, even where all of the technical developments have been favorable and where funding is seemingly non-limiting. In the future, duckweeds will probably find new uses in biotechnology whenever determined and competent scientists are met with favorable business conditions. So far that has not happened. This article has focused on just one of

27 Biolex Therapeutics Facility Expansion, Pittsboro, North Carolina, United States of America.

28 US NIH Clinical Trials.gov, Completed studies: NCT00863239, NCT00953589 and NCT00593151. URL: https://www.clinicaltrials.gov

29 Ariaans GJA et al. Expression of Secretory IgA Antibodies in Duckweed. US2014359902 (A1) — 2014-12-04.

several groups that have been working with duckweed technologies[30],[31],[32],[33],[34] which is not intended to slight other efforts, but rather to show the importance of critical business decisions in determining outcomes. The fundamental "charms" that made transgenic duckweeds so attractive as a platform technology still pertain. Furthermore the efforts of the team at Biolex and other companies have laid a strong and enduring foundation that can and should be exploited for profitable future biotechnologies.

30 Edelman; M. et al. Transgenic Lemnaceae. US Patent 7,176,352, February 13, 2007.

31 Honan, D. Duckweed: The Next Bio Fuel Revolution? Big Think. URL: http://bigthink.com/re-envision-toyota-blog/duckweed-the-next-bio-fuel-revolution

32 Yan Y, Candreva J, Shi H, Ernst E, Martienssen R, Schwender J, Shanklin J. Survey of the total fatty acid and triacylglycerol composition and content of 30 duckweed species and cloning of a Δ6-desaturase responsible for the production of γ-linolenic and stearidonic acids in *Lemna gibba*. BMC Plant Biol. 2013 Dec 5;13:201. doi: 10.1186/1471-2229-13-201. PubMed PMID: 24308551; PubMed Central PMCID: PMC3879013.

33 Cantó-Pastor A, Mollá-Morales A, Ernst E, Dahl W, Zhai J, Yan Y, Meyers BC, Shanklin J, Martienssen R. Efficient transformation and artificial miRNA gene silencing in *Lemna minor*. Plant Biol (Stuttg). 2015 Jan;17 Suppl 1:59-65. doi: 10.1111/plb.12215. Epub 2014 Jul 2. PubMed PMID: 24989135; PubMed Central PMCID: PMC4458260.

34 Firsov A, Tarasenko I, Mitiouchkina T, Ismailova N, Shaloiko L, Vainstein A, Dolgov S. High-Yield Expression of M2e Peptide of Avian Influenza Virus H5N1 in Transgenic Duckweed Plants. Mol Biotechnol. 2015 Mar 5. [Epub ahead of print] PubMed PMID: 25740321.

#	Patent #	Title	Patent type[35]
Patents assigned to NC State University			
1	46,281,374	Method for producing stably transformed duckweed using microprojectile bombardment	G
2	6,040,498	Genetically engineered duckweed	G
Patents assigned to Biolex			
1	6,815,184	Expression of biologically active polypeptide in duckweed	G
2	7,176,024	Bioreactor for growing biological materials supported on a liquid surface	T
3	7,326,385	Plate and method for high throughput screening	T
4	7,622,573	Expression control elements from the Lemnaceae family	G
5	7,632,983	Expression of monoclonal antibodies in duckweed	G
6	7,659,445	Expression of plasminogen and microplasminogen in duck weed	G
7	7,709,699	Use of duckweed in high throughput screening	T
8	7,884,264	Compositions and methods for inhibition of fucosyltransferase and xylosyltransferase expression in duckweed plants	G
9	7,959,910	C-terminally truncated interferon alpha variants	G
10	8,017,836	Expression of plasminogen and microplasminogen in duckweed	G
11	8,022,270	Expression of biologically active polypeptides in duckweed	G
12	8,034,916	Expression control elements from the Lemnaceae family	G
13	8,182,803	C-terminally truncated interferon alpha variants	G
14	8,394,384	Recombinant avian influenza vaccine and uses thereof	G
Patents invented at Biolex and assigned to Synthon			
1	8,716,55	Compositions and methods for inhibition of fucosyltransferase and xylosyltransferase expression in plants	G

35 Patent type: G = Genetic engineering of gene expression patents, T = Duckweed growth / screening technology patents

GreenOnyx

GreenOnyx is on a mission to make fresh nutrition-dense food an accessible global commodity while setting the bar on food supply sustainability.

Insufficient daily intake of fresh nutrition, mainly vegetables, is one of the 10 top factors for global mortality, while daily consumption of 200gr fresh vegetables has been proven to make a significant health impact.

GreenOnyx has selected Khai-Nam as it has been consumed for many generations in IndoChina, and its nutritional analysis show values equal to a mix of broccoli, kale and spinach. Since fresh Khai-Nam has a mild aroma, it easily blends in high portions within almost any dish or drink, adding the nutrients we crucially need without compromising taste or changing ones diet habits.

With a vision to provide fresh nutrients daily, GreenOnyx has developed a high-tech platform that can grow and deliver exceptional quantities of Khai-Nam, 365 days a year, anywhere, at a fixed low price. The solution packs the whole supply chain, from field to fork, into one compact automated system. It is designed to operate in-doors, and integrate into any food preparation environment while delivering fresh Khai-Nam at a push of a button.

In addition, GreenOnyx solution is poised to set the bar for food sustainability. It is free of produce waste as Khai-Nam is delivered "on-site" "per-demand" and is eaten as a whole. The system uses <5% of fresh water and fertilizers per kg, with a 1/3 of the carbon footprint, as compared to any state of the art agro-food practice. GreenOnyx does not use any pesticides, hormones or antibiotics. As important, it exploits existing urban infrastructures, thus does not need arable land or any new facility buildup.

Following an FDA clearance, based on the fact that GreenOnyx uses pure, natural and non-breaded strains, GreenOnyx has signed agreements with key food companies. Products that will be based on Khai-Nam will be available in the USA in the near future.

GreenOnyx is spearheading the edible awareness of Khai-Nam and elevating it to the mass public. We work closely with leading researchers in the Duckweed field and hope our pioneering work will be beneficial to all the Duckweed research and commercial community.

For more information, please visit our site http://www.greenonyx.biz or contact the company CTO and co-founder, Tsipi Shoham, Ph.D, at Tsipi@GreenOnyx.biz.

Useful Methods 2: Sterilization of Duckweed

by Klaus-J. Appenroth, University of Jena, Plant Physiology, Dornburger Str. 159, 07743 Jena, Germany. Email: Klaus.Appenroth@uni-jena.de.

Sterilization of duckweed plants is unavoidable in the lab because many physiological or toxicological properties were influence in an unknown way by microorganisms, be it bacteria or fungi. Thus, the first step isolating clones from natural populations is surface sterilization. Moreover, it happens again and again that an already sterile clone gets infected during handling, even in a safety hood. Wherever axenic clones were kept under in vitro cultivation conditions, sterilization belongs to the routine techniques.

Different species (genera of species) have different sensitivity toward bleaching agents usually used for sterilization. Most sensitive are *Wolffiella* species followed by *Wolffia* species. *Lemna* is much more resistant and *Landoltia* and *Spirodela* are the most resistant.

I learned this method from the late Elias Landolt, ETH Zurich during one of my visits in his lab. Before that we kept each clone in 5 copies excluding this way that the whole clone with all copies will be infected. Now we keep usually only two copies from each clone.

For surface disinfection, we put the plants in a plastic tube (Falcon), in dependence of the size of the plants having a volume of 15 ml or 50 ml. The commercial available "*Eau de Javel*" is diluted in water for different concentrations and the fronds are treated for different periods by gentle shaking:

Wolffia, *Wolffiella*: 1 – 3 %, 2, 3, 4, and 5 min

Lemna: 3 – 5 %, 3, 4, 5, and 6 min

Landoltia, *Spirodela*: 5 – 10 %, 3, 5, 7, and 9 min.

"Eau de Javel" is available at least in Switzerland and Germany (producer Floreal Haagen, Wadgassen, Germany). It contains 2.4 % NaOCl. This means that we sterilize *Landoltia* fronds in a 0.24 % solution of NaOCl using "Eau de Javel". Thereafter, plants were transferred into Erlenmeyer flasks with nutrient medium containing sugar (50 mM glucose or 25 mM sucrose) (e.g. 180 ml per flask) without further washing in sterile water. After a few days, better after 14 days, the medium remains clear when all bacteria and fungi are killed.

Originally, we washed the plants in 70 % ethanol before bathing them in "Eau de Javel" but it turned out that this does not improve the rate of success. At the end we have typically 4 Erlenmeyer flasks with fronds treated for different times in the bleaching bath. In the cultivation room at 25oC we follow the development of the plants in these Erlenmeyer flasks.

Sterilized plants can be transferred also on Agar in Petri dishes. The Agar-layer also has to contain sugar to control the success of the sterilization. In this case, some colonies might be unsterile without infecting sterile colonies in the same Petri dish. If this method is used, washing the fronds in sterile water after sterilization is indispensable in order to remove traces of the beaching solution.

We also used other commercially available preparations like “Danklorix” (CP GABA, Hamburg, Germany), available at least in Austria and Germany. This product contains 2.8 % NaOCl and can be used in the same way as “Eau de Javel”.

Limited experience exists with commercial available sodium hypochlorit (NaOCl) solution. However, the concentration is much higher (typically 12 %) and it has to be diluted accordingly. Moreover, as it is free of surfactants, it is useful to add a small amount of a mild detergent like 0.5 % of Triton X-100 to get the surface of the whole plant body wetted.

There are other beaching agents like “Lizol” (Reckitt Benckisier, Parwanno, Himachel Pradesh, India) having e.g. 0.6 % NaOCl. However in this case, a very high concentration of surfactants is included. We were not able to wash the surfactants properly away from the surface of the plants and the recovering rates were very low.

Experiments for Students part 2: Demonstrating the Effect of an Herbicide – SAN9789 (Norflurazon)

1. Introduction

Norflurazon belongs to the group of bleaching herbicides. Any other bleaching herbicide can also be used in this experiment. This experiment gives the opportunity to explain the complex effect of herbicides and their interaction with photosynthesis.

2. Material and methods

Materials and equipment:

Erlenmeyer flasks (100 ml), 2 per group of students, cotton wool stoppers, inoculation needle, measure (50 ml), autoclave.

Plant material:

Axenic culture of *Lemna*, e.g. *Lemna minor*, *Lemna gibba*, *Lemna aequinoctialis*, pre-cultivated for ca. 14 days. Other species of duckweed can also be used.

Chemicals:

Autotrophic nutrient medium, e.g. according to "Photophysiology of turion formation and germination in *Spirodela polyrhiza*." Appenroth, KJ; Teller, S.; Horn, M. BIOLOGIA PLANTARUM 38, 95-106 (1996).

Other nutrient media (Steinberg, Schenk-Hildebrandt or Hoagland) are also possible. Half of the nutrient medium should contain the solved herbicide. Norflurazon has to be pre-solved in a small amount of ethanol and stirred overnight in the nutrient medium.

3. Experimental procedure

50 ml nutrient medium with or without herbicide are filled into the Erlenmeyer flasks and some plants (e.g. 10 fronds, **not 10 colonies!**) are inoculated into the solutions. Take care that no traces of the herbicide-containing medium reach the herbicide-medium, do not use the same inoculation needle. The flasks were kept in a cultivation room or on a windowsill exposed to daylight. As control cultures can also kept in complete darkness. After 2 weeks, numbers of green and white fronds have to be counted (calculated growth rate!) and the fronds have to be evaluated.

4. Theoretical background

The fact of bleaching is often assumed to be caused by inhibition of chlorophyll biosynthesis. This is not correct. Bleaching herbicides inhibit synthesis of carotenoids. Carotenoids are required to protect chlorophylls by taking over the surplus energy in light. Carotenoids quench electronically excited chlorophylls by different mechanisms. Carotenoids are relatively stable in plant cells. As a

consequence already existing cells (fronds) are not bleached in light as they have sufficient carotenoids. However, newly formed cells (fronds) cannot synthesis carotenoids in the presence of the herbicide. Therefore, these fronds are bleached. Often it can be observed that one part of a frond (the old part) is still green, the younger part bleached. In darkness no bleaching affect is observed.

From the Data Base

Highlights

Engineering Corynebacterium crenatum to produce higher alcohols for biofuel using hydrolysates of duckweed (Landoltia punctata) as feedstock

Su, Haifeng; Jiang, Juan; Lu, Qiuli; et al.

Microbial Cell Factories 14: Article Number: 16 (2015)

Early trials have demonstrated great potential for the use of duckweed (family Lemnaceae) as the next generation of energy plants for the production of biofuels. Achieving this technological advance demands research to develop novel bioengineering microorganisms that can ferment duckweed feedstock to produce higher alcohols. In this study, we used relevant genes to transfer five metabolic pathways of isoleucine, leucine and valine from the yeast Saccharomyces cerevisiae into the bioengineered microorganism Corynebacterium crenatum. Experimental results showed that the bioengineered strain was able to produce 1026.61 mg/L of 2-methyl-1-butanol by fermenting glucose, compared to 981.79 mg/L from the acid hydrolysates of duckweed. The highest isobutanol yields achieved were 1264.63 mg/L from glucose and 1154.83 mg/L from duckweed, and the corresponding highest yields of 3-methyl-1-butanol were 748.35 and 684.79 mg/L. Our findings demonstrate the feasibility of using bioengineered C. crenatum as a platform to construct a bacterial strain that is capable of producing higher alcohols. We have also shown the promise of using duckweed as the basis for developing higher alcohols, illustrating that this group of plants represents an ideal fermentation substrate that can be considered the next generation of alternative energy feedstocks.

Genetic characterization and barcoding of taxa in the genera Landoltia and Spirodela (Lemnaceae) by three plastidic markers and amplified fragment length polymorphism (AFLP)

Bog, Manuela; Lautenschlager, Ulrich; Landrock, Maria F.; et al.

Hydrobiologia 749: 169-182 (2015)

Duckweeds, the fastest growing angiosperms, are gaining increasing attention with respect to their practical applications. Different clones of the same duckweed species vary in their physiological properties. Hence, screening of suitable clones of a species is very important. To enable the identification of clones, a clear taxonomic classification and barcoding at different taxonomic levels, i.e. genera, species, and clones is a pre-requisite. In the present project, we have focused on the genera Spirodela and Landoltia. Spirodela polyrhiza (L.) Schleid. (42 clones), Spirodela intermedia W. Koch (14 clones), and Landoltia punctata (G. Meyer) Les & Crawford (15 clones) were characterized using three plastidic sequences (rpl16, rps16, atpF-atpH) and AFLP fingerprinting. Genome size

determination showed significant differences between the two genera. The genetic variability is lowest in S. polyrhiza and highest in S. intermedia. Although the resolution of phenetic variability by AFLP fingerprinting is much higher than the sequence variation of the selected plastidic regions, not all clones could be identified unequivocally. However, without any exception, all clones were strictly categorized into the three species as defined by the morphological markers. The results do not justify the separation of some clones as Spirodela biperforata from S. intermedia.

Biotechnology

Natural variance in salt tolerance and induction of starch accumulation in duckweeds

Sree, K. Sowjanya; Adelmann, Kai; Garcia, Cyrus; et al.

Planta 241: 1395-1404 (2015)

Ten of 34 tested duckweed clones showed relatively higher salt tolerance. Salinity stress induced high level of starch accumulation in these clones, making them potential feedstock candidates for biofuel production. Duckweeds are promising as a new generation of crop plants that requires minimal input while providing fast biomass production. Two important traits of interest that can impact on the economic viability of this system are their sensitivity to salt and the starch content of the harvested duckweed. We have surveyed 33 strains of duckweed selected from across all 5 genera and amongst 13 species to quantify the natural variance of these traits. We found that there are large ranges of intraspecific variations in salt tolerance, while all species examined accumulated more starch in response to the initial stages of salt stress. However, the magnitude of the change in starch content varied widely between strains. Our results suggest that specific duckweed clones can be cultivated under relatively saline conditions, while increasing salt in the medium before harvesting could be used to increase starch in duckweed biomass for bioethanol production.

Engineering Corynebacterium crenatum to produce higher alcohols for biofuel using hydrolysates of duckweed (Landoltia punctata) as feedstock

Su, Haifeng; Jiang, Juan; Lu, Qiuli; et al.

Microbial cell factories 14: 2015-Dec (Epub 2015 Feb 07)

Early trials have demonstrated great potential for the use of duckweed (family Lemnaceae) as the next generation of energy plants for the production of biofuels. Achieving this technological advance demands research to develop novel bioengineering microorganisms that can ferment duckweed feedstock to produce higher alcohols. In this study, we used relevant genes to transfer five metabolic pathways of isoleucine, leucine and valine from the yeast Saccharomyces cerevisiae into the bioengineered microorganism Corynebacterium crenatum. Experimental results showed that the bioengineered strain was able to produce 1026.61mg/L of 2-methyl-1-butanol by fermenting glucose, compared to 981.79mg/L from the acid hydrolysates of duckweed. The highest isobutanol

yields achieved were 1264.63mg/L from glucose and 1154.83mg/L from duckweed, and the corresponding highest yields of 3-methyl-1-butanol were 748.35 and 684.79mg/L. Our findings demonstrate the feasibility of using bioengineered C. crenatum as a platform to construct a bacterial strain that is capable of producing higher alcohols. We have also shown the promise of using duckweed as the basis for developing higher alcohols, illustrating that this group of plants represents an ideal fermentation substrate that can be considered the next generation of alternative energy feedstocks.

Effects of dispersal on total biomass in a patchy, heterogeneous system: Analysis and experiment

Zhang, Bo; Liu, Xin; DeAngelis, D L; et al.

Mathematical Biosciences 264: 54-62 (2015)

An intriguing recent result from mathematics is that a population diffusing at an intermediate rate in an environment in which resources vary spatially will reach a higher total equilibrium biomass than the population in an environment in which the same total resources are distributed homogeneously. We extended the current mathematical theory to apply to logistic growth and also showed that the result applies to patchy systems with dispersal among patches, both for continuous and discrete time. This allowed us to make specific predictions, through simulations, concerning the biomass dynamics, which were verified by a laboratory experiment. The experiment was a study of biomass growth of duckweed (Lemna minor Linn.), where the resources (nutrients added to water) were distributed homogeneously among a discrete series of water-filled containers in one treatment, and distributed heterogeneously in another treatment. The experimental results showed that total biomass peaked at an intermediate, relatively low, diffusion rate, higher than the total carrying capacity of the system and agreeing with the simulation model. The implications of the experiment to dynamics of source, sink, and pseudo-sink dynamics are discussed.

Catalytic fast pyrolysis of duckweed: Effects of pyrolysis parameters and optimization of aromatic production

Liu, Guangyi; Wright, Mark M.; Zhao, Qingliang; et al.

Journal of Analytical and Applied Pyrolysis 112: 29-36 (2015)

Catalytic fast pyrolysis (CFP) of duckweed is a potential source for biorenewable gasoline and diesel fuel production, but its ability to produce aromatics has not been investigated in detail. In this study, we employed a micro-pyrolyzer coupled with gas chromatograph mass spectrometer, flame ionization detector, and a thermal conductivity detector to conduct CFP of duckweed (Lemna minor) with HZSM-5 catalyst and obtained promising yields of total aromatic hydrocarbon (TAH) and benzene, toluene, and xylene (BTX). In the operating conditions studied, pyrolysis temperatures and catalyst-to-biomass ratios (CBR) had significant effects on product yields and distributions. Duckweed CFP can be

optimized to maximize either the TAH yield or the output of the BTX components. Optimal operating conditions were determined based on response surface methodology (RSM) with a 4 x 5 full factorial design (FFD). The RSM predicted optimal TAH and xylene carbon yields to be 27.2 mol% and 6.2 mol% at 736 degrees C and 662 degrees C, respectively, with CBR of 16:1. Optimal benzene and toluene carbon yields of 5.5 mol% and 8.0 mol%, respectively, were obtained at 750 degrees C and CBR of 20:1.

Steam explosion pretreatment and enzymatic saccharification of duckweed (Lemna minor) biomass

Zhao, X.; Moates, G. K.; Wilson, D. R.; et al.

Biomass and Bioenergy 72: 206-215 (2015)

Our previous research has shown that duckweed is potentially an ideal feedstock for the production of biofuels because it can be effectively saccharified enzymatically. Here we report the results of experiments in which duckweed was pre-treated by steam explosion prior to enzyme digestion. A range of temperatures, from 130 to 230 degrees C with a fixed retention time of 10 mm, were employed. The best pretreatment conditions were 210 degrees C for 10 min; these conditions produced the highest amount of water-soluble material (70%), the greatest levels of starch solubilisation (21%) and hemicellulose and pectic polysaccharides degradation (60%). The use of these steam explosion conditions enabled large reductions in the concentrations of enzymes required for effective saccharification. The amount of Celluclast required was reduced from 100 U (4.35 FPU) g(-1) substrate to 20 U g(-1) substrate, and additional beta-glucosidase was reduced from 100 to 2 U g(-1) substrate

Ecology

The influence of duckweed species diversity on ecophysiological tolerance to copper exposure

Zhao, Zhao; Shi, Huijuan; Duan, Dongzhu; et al.

Aquatic Toxicology 164: 92-8 (2015)

In excess, copper is toxic to plants. In the plants, Landoltia punctata and Lemna minor grown in mixed and monoculture, the effects of exposure to varying concentrations of copper (0.01, 0.1, 0.5 and 1mgL(-1) Cu) for seven days were assessed by measuring changes in the chlorophyll, protein and malondialdehyde (MDA) content, catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity. According to results, Cu levels in plants increased with increasing Cu concentration. The level of photosynthetic pigments and crude proteins decreased only upon exposure to high Cu concentrations. However, the starch and malondialdehyde (MDA) content increased. These results suggested a stress alleviation that was possibly the result of antioxidants such as CAT and SOD, the activities of which increased with increasing Cu levels. APX activity increased in L. punctata, but

decreased in L. minor, under monoculture or mixed culture conditions. In addition, the duckweed in mixed culture exhibited increased antioxidant enzyme activities which provide increased resistance to copper in moderate copper concentrations. As the copper concentration increased, the duckweed in the mixed culture limited the uptake of copper to avoid toxicity.

Effects of heavy metals on ultrastructure and Hsp70 induction in Lemna minor L. exposed to water along the Sarno River, Italy

Basile, A.; Sorbo, S.; Cardi, M.; et al.

Ecotoxicology and Environmental Safety 114: 93-101 (2015)

The effects of freshwater pollution in the highly contaminated river Sarno (Campania, Southern Italy) have been evaluated using bags containing the aquatic plant Lemna minor (Lemnacea, Arales), in order to determine morpho-physiological modifications as a response to pollutants. The exposition of Lemna bags for 7 days on three different sites along the river path showed alterations in chloroplasts and vacuoles shape and organization. Moreover, some specimens were exposed in vitro at the same heavy metal (HM) concentrations measured in the polluted sites of the river, and compared with data from the bag experiment; to verify the dose and time dependent effects, samples were exposed to HM in vitro at concentrations ranging from 10(-6) to 10(-4) M up to 7 days. Transmission electron microscopy (TEM) observations on in vitro plants confirmed that ultrastructural alterations affected most of plastids and the shape of different subcellular structures, namely vacuoles; in in vitro stressed specimens, Heat Shock Proteins 70 (Hsp70) levels changed, in dependence of changing levels of HM measured in different sites along the river path. Thus L. minor exhibited a possible correlation between the levels of HM pollution and Hsp70 occurrence; interestingly, the data presented showed that copper specifically increased Hsp70 levels at concentrations detected in polluted river waters, whereas cadmium and lead did not; on the other side, the latter represent highly toxic elements when specimens were exposed to higher levels in vitro. The effects of specific elements in vitro are compared to those observed in bags exposed along the river path; thus results are examined in order to propose L. minor as an organism able to be utilized to monitor heavy metals pollution; the possibility of using Hsp70 s as specific markers of HM pollution is discussed.

Feed & Food

Effects of diets with fermented duckweed (Lemna sp.) on growth performance and gene expression in the Pacific white shrimp, Litopenaeus vannamei

del Carmen Flores-Miranda, Ma; Luna-Gonzalez, Antonio; Veronica Cortes-Espinosa, Diana; et al.

Aquaculture International 23: 547-561 (2015)

This study evaluated the effects of diets with fermented duckweed flour (Lemna sp.) (FDF) on growth performance and gene expression in Pacific white shrimp, Litopenaeus vannamei. Shrimp were cultured in an outdoor system during 50 days and fed diets containing 0, 5, 15, 25, and 35 % FDF

replacing fishmeal (FM) (diets D0, D5, D15, D25, and D35, respectively). At the end of the bioassay, shrimp survival was 100 % in all treatments and growth performance was significantly better than D0 (100 % FM), especially in diet D35 with 35 % FDF. The mRNA expression of trypsin, chymotrypsin, cathepsin B, heat shock protein 70 (Lvhsp70), and heat shock protein 90 (Lvhsp90) was significantly increased at the highest FDF concentrations in diets (D15, D25, and D35) as compared to D0. Dietary FDF affected the immune system of shrimp only in diets D5 (superoxide dismutase and lysozyme) and D15 (lysozyme) where mRNA expression was significantly higher than D0. FM can be replaced with up to 35 % FDF without adversely affecting the survival and growth performance of cultured shrimp. The inclusion of FDF in diets affected the expression of stress and digestive genes, but, in immune-related genes, the effect did not show a clear trend.

Interaction with other organisms

Spirodela polyrhiza stimulates the growth of its endophytes but differentially increases their fenpropathrin-degradation capabilities

Xu, Xing-Jian; Sun, Ji-Quan; Nie, Yong; et al.

Chemosphere 125: 33-40 (2015)

In situ remediation of organic contaminants via physical, chemical, and biological approaches is a practical technique for cleansing contaminated water and soil. In the present study, we showed that the three bacterial strains Pseudomonas sp. E1, Klebsiella terrigena E42, and Pseudomonas sp. E46, which can infect and colonize the aquatic plant Spirodela polyrhiza, utilize fenpropathrin as the sole carbon source for growth. S. polyrhiza helped enhance fenpropathrin degradation by E46 by 17.5%, only slightly improved fenpropathrin degradation by E42, and had no effect on strain E1. The application of plant exudates and extracts from fenpropathrin-unexposed/induced plants stimulated bacterial growth of the three strains, but resulted in differential fenpropathrin degradation, suggesting that not all plants and their endophytic bacteria are suitable for coupling phytoremediation and microbial-remediation. Moreover, addition of soil sediments to a microcosm not only stimulated the growth of strain E46 but also increased the rate of fenpropathrin degradation.

Microbial community and removal of nitrogen via the addition of a carrier in a pilot-scale duckweed-based wastewater treatment system

Zhao, Yonggui; Fang, Yang; Jin, Yanling; et al.

Bioresource Technology 179: 549-558 (2015)

Carriers were added to a pilot-scale duckweed-based (Lemna japonica 0223) wastewater treatment system to immobilize and enhance microorganisms. This system and another parallel duckweed system without carriers were operated for 1.5 years. The results indicated the addition of the carrier did not significantly affect the growth and composition of duckweed, the recovery of total nitrogen (TN), total phosphorus (TP) and CO2 or the removal of TP. However, it significantly improved the

removal efficiency of TN and NH+4-N (by 19.97% and 15.02%, respectively). The use of 454 pyrosequencing revealed large differences of the microbial communities between the different components within a system and similarities within the same components between the two systems. The carrier biofilm had the highest bacterial diversity and relative abundance of nitrifying bacteria (3%) and denitrifying bacteria (24% of Rhodocyclaceae), which improved nitrogen removal of the system. An efficient N-removal duckweed system with enhanced microorganisms was established.

Draft Genome Sequence of Acinetobacter calcoaceticus Strain P23, a Plant Growth-Promoting Bacterium of Duckweed

Sugawara, Masayuki; Hosoyama, Akira; Yamazoe, Atsushi; et al.

Genome Announcements 3 Issue: 1 Published: 2015 Feb 26

Acinetobacter calcoaceticus strain P23 is a plant growth-promoting bacterium, which was isolated from the surface of duckweed. We report here the draft genome sequence of strain P23. The genome data will serve as a valuable reference for understanding the molecular mechanism of plant growth promotion in aquatic plants.

Molecular Biology

Uniconazole-induced starch accumulation in the bioenergy crop duckweed (Landoltia punctata) II: transcriptome alterations of pathways involved in carbohydrate metabolism and endogenous hormone crosstalk

Liu, Yang; Fang, Yang; Huang, Mengjun; et al.

Biotechnology for Biofuels 8, Article Number: 64 (2015)

Landoltia punctata is a widely distributed duckweed species with great potential to accumulate enormous amounts of starch for bioethanol production. We found that L. punctata can accumulate starch rapidly accompanied by alterations in endogenous hormone levels after uniconazole application, but the relationship between endogenous hormones and starch accumulation is still unclear. After spraying fronds with 800 mg/L uniconazole, L. punctata can accumulate starch quickly, with a dry weight starch content of up to 48% after 240 h of growth compared to 15.7% in the control group. Electron microscopy showed that the starch granule content was elevated after uniconazole application. The activities of key enzymes involved in starch synthesis were also significantly increased. Moreover, the expression of regulatory elements of the cytokinin (CK), abscisic acid (ABA) and gibberellin (GA) signaling pathways that are involved in chlorophyll and starch metabolism also changed correspondingly. Importantly, the expression levels of key enzymes involved in starch biosynthesis were up-regulated, while transcript-encoding enzymes involved in starch degradation and other carbohydrate metabolic branches were down-regulated. The increase of endogenous ABA and CK levels positively promoted the activity of ADP-glucose pyrophosphorylase (AGPase) and chlorophyll content, while the decrease in endogenous GA levels inactivated a-amylase. Thus, the

alterations of endogenous hormone levels resulted in starch accumulation due to regulation of the expression of genes involved in the starch metabolism pathway.

Uniconazole-induced starch accumulation in the bioenergy crop duckweed (Landoltia punctata) I: transcriptome analysis of the effects of uniconazole on chlorophyll and endogenous hormone biosynthesis

Liu, Yang; Fang, Yang; Huang, Mengjun; et al.

Biotechnology for Biofuels 8: Article Number 57 (2015)

Duckweed is a novel aquatic bioenergy crop that is found ubiquitously throughout the world. Uniconazole plays an important role in improving crop production through the regulation of endogenous hormone levels. We found that a high quantity and quality of duckweed growth can be achieved by uniconazole application, although the mechanisms are unknown. The fronds of Landoltia punctata were sprayed evenly with 800 mg/L uniconazole. The dry weight following treatment increased by 10% compared to the controls at 240 h. Endogenous cytokinin (CK) and abscisic acid (ABA) content both increased compared to the control, while the level of gibberellins (GAs) decreased. Additionally, gene expression profiling results showed that the expression of transcripts encoding key enzymes involved in endogenous CK and ABA biosynthesis were up-regulated, while the transcripts of key enzymes for GAs biosynthesis were down-regulated. On the other hand, chlorophyll a and chlorophyll b contents were both increased compared with the control. Moreover, the net photosynthetic rate was elevated to 25.6 mu mol CO2/m(2)/s compared with the control value of 22.05 mu mol CO2/m(2)/s. Importantly, the expression of some chlorophyll biosynthesis-related transcripts was up-regulated. Uniconazole treatment altered endogenous hormone levels and enhanced chlorophyll content and net photosynthetic rate in duckweed by regulating key enzymes involved in endogenous hormone and chlorophyll biosynthesis. The alterations of endogenous hormones and the increase of chlorophyll and photosynthetic rate data support the increase of biomass and starch accumulation.

Physiology

Senescence in duckweed: age-related declines in survival, reproduction and offspring quality

Barks, Patrick M.; Laird, Robert A.

Functional Ecology 29: 540-548 (2015)

As they grow old, most organisms experience progressive physiological deterioration resulting in declining rates of survival and reproduction – a seemingly maladaptive phenomenon known as senescence. Although senescence is usually defined with respect only to survival and reproduction, a third component of fitness, offspring quality, may also decline with age. Few studies, however, have assessed age-related changes in offspring quality using measures that truly reflect fitness. In a

controlled environment, we tested for age-related declines in three demographic components of fitness (survival, reproduction and offspring quality) in Lemna minor, a small aquatic plant in the subfamily Lemnoideae (the duckweeds) with a short life span and rapid rate of asexual reproduction. Our primary measure of offspring quality, the intrinsic rate of increase, more closely approximates fitness than measures used in previous studies such as size, life span and total reproductive output. We observed strong age-related declines in all three components of fitness: old plants had lower rates of survival and reproduction, and produced lower-quality offspring than younger plants. Theoretical and empirical research on the evolutionary biology of senescence should devote more attention to offspring quality. This often unrecognized component of fitness may change with age - as we have shown in L.minor - and may be shaped by, and feed back into, the same evolutionary forces that give rise to senescence.

Geomagnetic and Strong Static Magnetic Field Effects on Growth and Chlorophylla Fluorescencein Lemna minor

Jan, Luka; Fefer, Dusan; Kosmelj, Katarina; Gaberscik, Alenka; Jerman, Igor

Bioelectromagnetics 36: 190-203 (2015)

The geomagnetic field (GMF) varies over Earth's surface and changes over time, but it is generally not considered as a factor that could influence plant growth. The effects of reduced and enhanced GMFs and a strong static magnetic field on growth and chlorophyll a (Chl a) fluorescence of Lemna minor plants were investigated under controlled conditions. A standard 7 day test was conducted in extreme geomagnetic environments of 4 mu T and 100 mu T as well as in a strong static magnetic field environment of 150mT. Specific growth rates as well as slow and fast Chl a fluorescence kinetics were measured after 7 days incubation. The results, compared to those of controls, showed that the reduced GMF significantly stimulated growth rate of the total frond area in the magnetically treated plants. However, the enhanced GMF pointed towards inhibition of growth rate in exposed plants in comparison to control, but the difference was not statistically significant. This trend was not observed in the case of treatments with strong static magnetic fields. Our measurements suggest that the efficiency of photosystem II is not affected by variations in GMF. In contrast, the strong static magnetic field seems to have the potential to increase initial Chl a fluorescence and energy dissipation in Lemna minor plants.

Effects of Superparamagnetic Iron Oxide Nanoparticles on Photosynthesis and Growth of the Aquatic Plant Lemna gibba

Barhoumi, Lotfi; Oukarroum, Abdallah; Ben Taher, Lotfi; et al.

Archives of Environmental Contamination and Toxicology 68: 510-520 (2015)

Toxicity of superparamagnetic iron oxide nanoparticles (SPION) was investigated in Lemna gibba plants exposed for 7 days to Fe3O4 (SPION-1), Co0.2Zn0.8Fe2O4 (SPION-2), or Co0.5Zn0.5Fe2O4

(SPION-3) at 0, 12.5, 25, 50, 100, 200 or 400 A mu g mL(-1). At < 400 A mu g mL(-1) of SPION exposure, toxicity was indicated by decrease of chlorophyll content, deterioration of photosystem II (PSII) functions, strong production of reactive oxygen species (ROS), and inhibition of growth rate based on fresh weight (52-59 %) or frond number (32-49 %). The performance index of PSII activity was the most sensitive biomarker of PSII functions and decreased by 83, 86, and 79 % for SPION-1, SPION-2, and SPION-3, respectively. According to the change of these biomarkers, the exposure of SPION suspensions to L. gibba caused several alterations to the entire plant cellular system, which may come from both the uptake of nanoparticles and metal ions in the soluble fraction. Our results, based on the change of several biomarkers, showed that these SPION have a complex toxic mode of action on the entire plant system and therefore affects its viability. Therefore, the plant model L. gibba was shown to be a sensitive bioindicator of SPION cellular toxicity and thus can be used in the development of a laboratory bioassay toxicity testing.

Phytoremediation

Use of duckweed-based constructed wetlands for nutrient recovery and pollutant reduction from dairy wastewater

Adhikari, Umesh; Harrigan, Timothy; Reinhold, Dawn M.

Ecological Engineering 78: 6-14 (2015)

Over the last few decades, constructed wetlands have increasingly been designed and implemented to treat agricultural wastewaters. However, while treatment of manure-containing wastewaters protects water quality, it also eliminates a valuable source of nutrient-laden soil amendments. The goal of this research is to investigate the ability of wetlands to simultaneously treat high-strength manure-containing wastewaters while recovering nutrients from the manures in plant biomass. Diluted raw dairy waste was fed to a combination of duckweed-based surface flow and subsurface flow wetlands. Nutrient recovery through duckweed harvesting and waste treatment, characterized by chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and E. coli removal, were assessed under steady-state conditions for three strengths of waste. Duckweed-based wetlands used for primary treatment had higher removal rates of COD and TN than those used for secondary treatment; however, no significant difference was observed for TP removal rates. COD removal ranged from 3 to 81% in primary duckweed wetlands and from -35% to 38% in secondary duckweed wetlands. Areal removal rates for nutrients in primary duckweed wetlands were 194.9 +/- 18.9 gTN/m(2)/yr and 13.0 +/- 3.0 g TP/m(2)/yr, while removal rates in secondary duckweed wetlands were 104.1 +/- 13.1 gTN/m(2)/yr and 9.3 +/- 2.1 g TP/m(2)/yr. Removal of COD, TN, and TP more closely followed first-order removal to a background concentration than first-order removal or a previously published model for duckweed ponds. Mean log E. coli reduction of 0.30 obtained in this experiment was within the range reported in literature. Duckweed production in the wetlands was satisfactorily described as a second-order polynomial function of influent TN concentration. More N and P was recovered from primary wetlands as compared to secondary wetlands. Average N and P recovered by harvesting duckweed across all the wetlands were 22.4 gN/m(2)/yr and 7.4 P/m(2)/yr, respectively.

Phytoremediation Potential of Duckweed (Lemna minor L.) On Steel Wastewater

Saha, Priyanka; Banerjee, Angela; Sarkar, Supriya

International Journal of Phytoremediation 17: 589-596 (2015)

An eco-friendly and cost effective technique-phytoremediation was used to remediate contaminants from waste water. This study demonstrated that phytoremediation ability of duckweed (Lemna minor L.) to remove chloride, sulphate from Biological Oxygen Treatment (BOT) waste water of coke oven plant. The BOT water quality was assessed by analyzing physico-biochemical characters-pH, Biological oxygen demand (BOD), Chemical oxygen demand (COD), total dissolved solids (TDS) and elemental concentration. It was observed that an increase in pH value indicated an improvement of water quality. The experimental results showed that, duckweed effectively removed 30% chloride, 16% sulphate and 14% TDS from BOT waste water, which suggested its ability in phytoremediation for removal of chloride and sulphate from BOT waste water. A maximum increase of 30% relative growth rate of duckweed was achieved after 21 days of experiment. Thus, it was concluded that duckweed, an aquatic plant, can be considered for treatment of the effluent discharged from the coke oven plant.

Phytotoxicity

Comparative study on the sensitivity of turions and active fronds of giant duckweed (Spirodela polyrhiza (L.) Schleiden) to heavy metal treatments

Olah, Viktor; Hepp, Anna; Meszaros, Ilona

Chemosphere 132: 40-46 (2015)

Standard ecotoxicological test procedures use only active forms of aquatic plants. The potential effects of toxicants on vegetative propagules, which play an important role in the survival of several aquatic plant species, is not well understood. Because turion-like resting propagules overwinter on the water bottom in temperate regions, they could be exposed to contaminants for longer periods than active plants. Due to its turion producing capability, giant duckweed (Spirodela polyrhiza) is widely used in studying morphogenesis, dormancy, and activation mechanisms in plants. It is also suitable for ecotoxicological purposes. The present work aims to compare the growth inhibition sensitivity of active (normal frond) and overwintering (turion) forms of S. polyrhiza to concentrations of nickel (Ni), cadmium (Cd) and hexavalent chromium (Cr) ranging from 0 to 100mgL(-1). The results indicated that in general, resting turions have higher heavy metal tolerance than active fronds. Cd proved to be the most toxic heavy metal to S. polyrhiza active frond cultures because it induced rapid turion formation. In contrast, the toxicity of Ni and Cr were found to be similar but lower than the effects of Cd. Cr treatments up to 10mgL(-1) did not result in any future negative effects on turion activation. Turions did not survive heavy metal treatments at higher concentrations of Cr. Cd and Ni treatments affected both the floating-up and germination of turions but did not significantly affect the vigor of sprouts. Higher concentrations (of 100mgL(-1)) Cd completely inhibited germination.

Phytotoxicity of cobalt ions on the duckweed Lemna minor - Morphology, ion uptake, and starch accumulation

Sree, K. Sowjanya; Keresztes, Aron; Mueller-Roeber, Bernd; et al.

Chemosphere 131: 149-156 (2015)

Cobalt (Co2+) inhibits vegetative growth of Lemna minor gradually from 1 mu M to 100 mu M. Fronds accumulated up to 21 mg Co2+ g(-1) dry weight at 10 mu M external Co2+ indicating hyperaccumulation. Interestingly, accumulation of Co2+ did not decrease the iron (Fe) content in fronds, highlighting L. minor as a suitable system for studying effects of Co2+ undisturbed by Fe deficiency symptoms unlike most other plants. Digital image analysis revealed the size distribution of fronds after Co2+ treatment and also a reduction in pigmentation of newly formed daughter fronds unlike the mother fronds during the 7-day treatment. Neither chlorophyll nor photosystem II fluorescence changed significantly during the initial 4 d, indicating effective photosynthesis. During the later phase of the 7-day treatment, however, chlorophyll content and photosynthetic efficiency decreased in the Co2+-treated daughter fronds, indicating that Co2+ inhibits the biosynthesis of chlorophyll rather than leading to the destruction of pre-existing pigment molecules. In addition, during the first 4 d of Co2+ treatment starch accumulated in the fronds and led to the transition of chloroplasts to chloro-amyloplasts and amylo-chloroplasts, while starch levels strongly decreased thereafter.

Comparative toxicity of copper nanoparticles across three Lemnaceae species

Song, Lan; Vijver, Martina G.; Peijnenburg, Willie J. G. M.

Science of the Total Environment 518: 217-224 (2015)

Metallic nanoparticles can end up in aquatic ecosystems due to their widespread application. Even though the toxicological effects of metallic nanoparticles to a diversity of species have been reported extensively, the toxicological data achieved in different studies are not always comparable and little is known regarding the comparative toxicity of nanoparticles across species, as different test strategies and endpoints were applied. To attempt to fill this knowledge gap, Spirodela polyrhiza, Lemna minor and Wolffia arrhiza were exposed to 25 nm spherical copper nanoparticles to investigate the inhibiting effect of copper nanoparticle suspensions across species at three endpoints: total frond area, frond number and dry weight based relative growth rate. The total frond area based relative growth rate was found to be the most sensitive endpoint, with an EC50 of 1.15 +/- 0.09 mg/L for S. polyrhiza, 0.84 +/- 0.12 mg/L for L. minor and 0.64 +/- 0.05 mg/L for W. arrhiza. Both the particles and the copper ions contributed to the inhibiting effects of copper nanoparticle suspensions at all endpoints studied. Dose-response related inhibiting effects caused by the copper ions were found at all endpoints studied, whereas the particles only showed dose-response related inhibiting effects on the total frond area based relative growth rate. This suggests that different physiological processes

are involved in case of exposure to particles and copper ions. W. arrhiza was found to be the most sensitive species tested and S. polyrhiza was the least sensitive species tested, when the inhibiting effect was evaluated based on the relative growth rate calculated from total frond area. These findings exemplify the importance of identifying the suitable endpoints of toxicity assessment and considering the intrinsic differences between species when evaluating the toxicological profile of metallic nanoparticles across species.

Quantitative structure-activity relationship (QSAR) prediction of (eco)toxicity of short aliphatic protic ionic liquids

Peric, Brezana; Sierra, Jordi; Marti, Esther; et al.

Ecotoxicology and Environmental Safety 115: 257-262 (2015)

Ionic liquids (ILs) are considered as a group of very promising compounds due to their excellent properties (practical non-volatility, high thermal stability and very good and diverse solving capacity). The ILs have a good prospect of replacing traditional organic solvents in vast variety of applications. However, the complete information on their environmental impact is still not available. There is also an enormous number of possible combinations of anions and cations which can form ILs, the fact that requires a method allowing the prediction of toxicity of existing and potential ILs. In this study, a group contribution QSAR model has been used in order to predict the (eco)toxicity of protic and aprotic ILs for five tests (Microtox(R), Pseudokirchneriella subcapitata and Lemna minor growth inhibition test, and Acetylcholinestherase inhibition and Cell viability assay with IPC-81 cells). The predicted and experimental toxicity are well correlated. A prediction of EC50 for these (eco)toxicity tests has also been made for eight representatives of the new family of short aliphatic protic ILs, whose toxicity has not been determined experimentally to date. The QSAR model applied in this study can allow the selection of potentially less toxic ILs amongst the existing ones (e.g. in the case of aprotic ILs), but it can also be very helpful in directing the synthesis efforts toward developing new "greener" ILs respectful with the environment (e.g. short aliphatic protic ILs).

Response of duckweed to various concentrations of selenite

Mechora, Spela; Stibilj, Vekoslava; Germ, Mateja

Environmental Science and Pollution Research 22: 2416-2422 (2015)

The uptake of Se(IV) and its effects on the physiological and biochemical characteristics of duckweed (Lemna minor L.) have been studied. Duckweed plants were cultivated in controlled conditions for 7 weeks in different concentrations of Na selenite: 0.5, 1, 2, 5 (exposed 42 days) and 10 mg Se L-1 (survived 7-21 days). The addition of 1 mg Se L-1 did not negatively affect photochemical efficiency whilst respiratory potential increased in weeks 2-4 compared to control. The addition of 1 mg Se(IV) L-1 increased the amount of chlorophyll a in weeks 3 and 4 and the amount of carotenoids in weeks 1, 3 and 5. Concentrations of 2 and 5 mg Se L-1 negatively affected photochemical efficiency in

weeks 3 and 4, and increased respiratory potential in comparison to the control in weeks 1–4, whilst beyond week 4, the respiratory potential decreased. Plants exposed to the highest concentration of Se(IV) had to be replaced twice during the experiment because they were dying. That was reflected in photochemical efficiency as well as in respiratory potential, which decreased in time. The content of Se in duckweed increased with the increasing concentration of Se: plants growing in 0.5 mg Se L−1 contained 0.9 mg Se g(−1) DM and plants exposed to 5 mg Se L−1 contained 5.8 mg Se g(−1) DM. The group of plants exposed to 10 mg Se L−1 for 21 days contained 19.5 mg Se g(−1) DM. Our study revealed that duckweed absorbed high amount of Se(IV) from the water.

Taxonomy and Systematics

Genetic characterization and barcoding of taxa in the genera Landoltia and Spirodela (Lemnaceae) by three plastidic markers and amplified fragment length polymorphism (AFLP)

Bog, Manuela; Lautenschlager, Ulrich; Landrock, Maria F.; et al.

Hydrobiologia 749: 169–182 (2015)

Duckweeds, the fastest growing angiosperms, are gaining increasing attention with respect to their practical applications. Different clones of the same duckweed species vary in their physiological properties. Hence, screening of suitable clones of a species is very important. To enable the identification of clones, a clear taxonomic classification and barcoding at different taxonomic levels, i.e. genera, species, and clones is a pre-requisite. In the present project, we have focused on the genera Spirodela and Landoltia. Spirodela polyrhiza (L.) Schleid. (42 clones), Spirodela intermedia W. Koch (14 clones), and Landoltia punctata (G. Meyer) Les & Crawford (15 clones) were characterized using three plastidic sequences (rpl16, rps16, atpF-atpH) and AFLP fingerprinting. Genome size determination showed significant differences between the two genera. The genetic variability is lowest in S. polyrhiza and highest in S. intermedia. Although the resolution of phenetic variability by AFLP fingerprinting is much higher than the sequence variation of the selected plastidic regions, not all clones could be identified unequivocally. However, without any exception, all clones were strictly categorized into the three species as defined by the morphological markers. The results do not justify the separation of some clones as Spirodela biperforata from S. intermedia.

Links for Further Reading

http://www.duckweed2015.cosmos.bot.kyoto-u.ac.jp Duckweed 2015 Conference site- watch for details as Kyoto, Japan conference preparations develop.

http://duckweed2013.rutgers.edu/ Past duckweed conference papers and proceedings held at Rutgers University, New Brunswick, NJ in Aug, 2013

http://Lemnapedia.org Online developing compendium of duckweed research & applications, founded by the ISCDRA.

http://InternationalLemnaAssociation.org Working to develop commercial applications for duckweed globally, Exec. Director, Tamra Fakhoorian

http://www.mobot.org/jwcross/duckweed/duckweed.htm Comprehensive site on all things duckweed-related, By Dr. John Cross.

http://plants.ifas.ufl.edu/ University of Florida's Center for Aquatic & Invasive Plants

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