国際シンポジウム

藻類バイオ燃料の実用化に向けて
International Symposium on Algal Biofuels

2011年11月17日［木］

共催
農林水産技術会議事務局
中央大学研究開発機構

文京シビックセンター　小ホール

http://www.bio.chuo-u.ac.jp/harayama/1117symposium.html

革新的なＣＯ$_2$高吸収バイオマスの利用技術の開発

農林水産技術会議プロジェクトの概要

近年、化石燃料の枯渇やCO_2排出による地球温暖化といった観点から、カーボンニュートラルなエネルギーとしてバイオマス燃料の利用が重要視されています。 微細藻類は高等植物と同様にCO_2、光エネルギー、水を用いて光合成を行います。その微細藻類の中には、単位面積あたりのCO_2固定速度が高等植物の10倍以上のものがおり、バイオ燃料の有力な担い手と考えられます。

しかし、藻類の大量培養や回収などの技術が未熟なことから、微細藻類を用いた現状での燃料生産コストは非常に高く、商業ベースとして成り立ちません。 微細藻類バイオマスの利活用を行うには、バイオマス生産（藻類培養）から中間処理（回収、油脂抽出）を経て最終生成品製造に至るまでの各工程をできるだけ簡素なものにして、 低コスト化を実現し、さらに、抽出残渣についても有効利用を図る必要があると考えられます。 そこで、本プロジェクト研究グループでは、主に軽油生産性能力を有する単細胞緑藻*Pseudochoricystis ellipsoidea*を活用し、微細藻類バイオマスを利用するためのトータルプロセスの開発を行っています。 この目的を実現するために、大きく分けて、

（a）微細藻類バイオマス生産および中間処理コストの削減
（b）微細藻類バイオマスの利活用の拡大を図る開発研究
（c）微細藻類を屋外で大量培養した場合の環境への影響

の3つの観点から研究を実施しています。

プロジェクト研究について

本プロジェクト研究は、中央大学を中心とする9機関(国立大学法人京都大学、株式会社デンソー、マイクロアルジェコーポレーション株式会社、 学校法人中部大学、トヨタ自動車株式会社、株式会社豊田中央研究所、国立大学法人佐賀大学、国立大学法人お茶の水女子大学)が、 農林水産省の実施する「地域活性化のためのバイオマス開発委託事業」の委託先に採択され、農林水産省からの委託プロジェクト研究の一環として、平成22年度から実施されているものです。 このプロジェクトでは、*P. ellipsoidea*あるいは新規に分離する高油脂蓄積微細藻類を活用し、微細藻類バイオマスの高効率生産から利活用までの一連のプロセスを開発し、 地域活性化のためのバイオマス利活用モデルを構築することを目的としています。

本シンポジウム開催について

このたび、上記プロジェクト研究の研究成果を発表することを目的に、農林水産技術会議事務局並びに中央大学研究開発機構の共催で開催することとなりました。 当該事業の参画機関による発表並びにポスター展示と併せて、海外の研究者を招聘し、先進的な研究動向について講演を行うとともに、この分野の研究に関わる国内研究者によるポスター展示を行います。

本シンポジウムが研究交流の場となり、ひいてはこの分野の研究の推進に繋がることを期待しています。

プログラム
Program

13:00　開会の辞
(農林水産省　農林水産技術会議事務局　研究開発官　齋藤 伸郎)

＜第1部テーマ：微細藻類培養テクノロジー＞

13:10-13:50　Maria J. Barbosa, Food and Biobased Research, Wageningen University and Research Center
"**An outlook on microalgal biofuels" …… 3**

13:55-14:15　Hiroaki Fukuda, DENSO CORPORATION Research laboratories
"**Open raceway ponds cultivation of *Pseudochoricystis ellipsoidea"*…… 4**

14:20-14:40　Norihide Kurano, DENSO CORPORATION Research laboratories
"**Quest for microalgal strains suitable for outdoor mass culture" …… 5**

＜POSTER SESSION＞

14:50-16:10　Poster Session・Coffee **…… 9**

＜第2部テーマ：微細藻類の育種と安全性＞

16:15-16:35　Misako Kato, Graduate School of Humanities and Sciences, Ochanomizu University
"**Human health risk assessment and potential harm to natural habitats associated with large-scale algae cultivation" …… 6**

16:40-17:10　Shigeaki Harayama, Department of Biological Science, Chuo University
"**Breeding better "energy algae"" …… 7**

17:15-17:55　Qiang Hu, Laboratory for Algae Research and Biotechnology, Arizona State University
"Toward understanding molecular and cellular mechanisms of lipid synthesis and accumulation in microalgae and implications for algal biofuels production" …… 8

17:55　閉会の辞
(中央大学研究開発機構　機構長　齋藤 邦夫)

18:30-20:00　懇親会

26階椿山荘にて懇親会を行います(会費:5000円)。奮ってご参加下さい。

当日のご参加も受付けています。

Invited Speaker
Profile

Dr. Maria .J. Barbosa

AlgaePARC, Wageningen University and Research Center

Dr. Maria J. Barbosa obtained her Master in Science degree from the Catholic University, Porto, Portugal, and her Ph.D. degree from the Wageningen University, The Netherlands. Her research involved in the scaled-up production and optimization of microalgal cultivation with photobioreactors. She is currently associated with Food and Biobased Research where she is developing technologies for economically viable large scale algal cultivation for the production of valuable bioproducts.

She is also an advisor of the IIMSAM (Inter-Governmental Institution for the use of Microalgae Spirulina Against Malnutrition), bringing her expertise to the organization in the development of photobioreactors for high-yield cultivation of Spirulina which will be used as a human dietary supplement to improve malnutrition of people in developing countries.

She is a winner of the European Innovation Award 2005 from CSK Food Enrichment. Her research works have been published internationally. For example, she is the coauthor of the Science Magazine article [Wijffels RH, Barbosa MJ.An outlook on microalgal biofuels. (2010) Science 329:796-799.], and the booklet “Microalgae: green gold of the future?”, published by Wageningen UR Food & Biobased Research (http://www.groenegrondstoffen.nl/downloads/Boekjes/12Microalgae_UK.pdf).

She is Director of AlgaePARC (Algae Production & Research Centre), a new pilot plant facility dedicated to applied research on microalgae biotechnology in which a 5 year research program has recently started in collaboration with 18 Industrial partners. The aim is to decrease production costs, decrease energy requirements and to obtain reliable information for the scale-up of microalgae plants.

Invited Speaker
Profile

Dr. Qiang Hu

Arizona State University, School of Life Sciences

Dr. Qiang Hu is a Professor and co-Director of the Laboratory for Algae Research and Biotechnology at Arizona State University. He is also a co-director of Arizona Center for Algae Technology and Innovation. Dr Hu has 25+ years of experience in fundamental and applied research on algae in topics ranging from photosynthesis, biosynthesis of lipids and carotenoids, growth physiology of high-density algal culture, photobioreactor system design, and application of algal mass culture technology for biofuels and chemicals, and for environmental bioremediation. He has authored or co-authored numerous research articles and book chapters and is the inventor or co-inventor on 24 patents and patent disclosures. Dr. Hu has served on a number of review panels for the DOE, NSF, EPA, and NOAA biomass and bioenergy programs and was a contributor to the U.S. DOE National Algal Biofuels Technology Roadmap.

Dr. Hu received a Bachelors of Science in Biology from Hubei University and a Masters of Science in Hydrobiology from the Chinese Academy of Sciences in China, and a Ph.D. degree in Microalgal Biotechnology from the Ben-Gurion University of the Negev in Israel.

口頭発表
Oral Presentation

An outlook on Microalgal Biofuels

Maria J. Barbosa & René Wijffels

Wageningen University and Research Center, Food and Biobased Research, Post Office Box 17, 6700 AA Wageningen, Netherlands
E-mail: maria.barbosa@wur.nl

Our goal is to develop sustainable biobased production strategies in which phototrophic microalgae are used for a single step conversion of light energy into functional products without depletion of natural resources.

Microalgae are considered one of the most promising feedstocks for sustainable production of biofuels. Microalgae do not need to be grown in agricultural areas (surface areas not suitable for agriculture can be used as well), can be grown on seawater (in addition to fresh water), can be grown on residual nutrients, have a high areal productivity and via biorefinery the algal biomass can be fractionated into valuable products (e.g. food proteins, starch, lipids for biofuels). The technology for production is still immature, but if developed it is expected that biomass can be produced at commercial scale for a cost price less than 0,50 €/kg dry biomass.

Wageningen UR believes this can be realized and we developed projects in recent years which allowed the build-up of critical mass and infrastructure to become a leading R&D player in this field. The research program is multidisciplinary; fundamental biological aspects, bioprocess engineering, scale up, biorefinery, chain development and systems analysis are studied in an integrated way. This multidisciplinary approach gave us a unique position and for that reason we have been able to develop innovative projects with many of the internationally leading companies in this field.

Recently we started the project AlgaePARC (Algae Production and Research Centre) with 18 industrial partners. AlgaePARC is a pilot facility with which we intend to bridge the gap between basic research and demonstration projects. In AlgaePARC we will compare state of the art technologies and develop new reactor concepts, aiming at reducing production costs, developing process control strategies, reducing energy input and gathering reliable information for scale-up.

In this presentation, I will give first a general overview of our research activities in the field of microalgae at Wageningen UR, focus on AlgaePARC and finish with an outlook on our future research activities.

口頭発表
Oral Presentation

Open raceway ponds cultivation of *Pseudochoricystis ellipsoidea*

Hiroaki Fukuda

DENSO CORPORATION, Research laboratories (HIROAKI_FUKUDA@denso.co.jp)

Currently, microalgae are recognized as a resource of second generation biofuels. The main advantages of the microalgae are that they have a higher photon conversion efficiency (higher biomass yield) compared with terrestrial plants, and that they do not compete with food crops.

Since July 2008, DENSO has been involved in the research on two kinds of microalgae *Botryococcus* and *Pseudochoricystis*. *Pseudochoricystis* is easy to cultivate in open ponds, because it can grow under acidic condition. We had constructed pilot scale raceway ponds (two 20 m^2 and three 60 m^2 ponds) in our Zenmyo factory on June 2010. We have been cultivating *Pseudochoricystis* in the raceway ponds and could capture the CO_2 in the flue gas from a cogeneration power system in the factory. The effluent from a wastewater treatment plant in the factory have been reused as water resource to cultivate microalgae, while the exhausted vapor from the cogeneration power system is reused as a heat source to dry the harvested cells.

We have started the MAFF project since July 2010 and have improved several process of biofuels production to reduce the production cost. In this presentation, I will introduce the remarkable ability of *Pseudochoricystis* to grow in open ponds and to produce biofuels.

口頭発表
Oral Presentation

Quest for microalgal strains suitable for outdoor mass culture

Norihide Kurano[1], Hideaki Miyashita[2]

1 DENSO CORPORATION, Research Laboratories (norihide_kurano@denso.co.jp)
2 Graduate School of Human and Environmental Studies, Kyoto University
(miyashita@hm1.mbox.media.kyoto-u.ac.jp)

Selection of microalgal strains which are suitable for outdoor mass cultures is one of the most important factors for successful algae-based biofuels production. Although it is known that so many kinds of microalgal species exist, only a few can be cultured in very large scale open ponds. Fortunately, "*Pseudochoricystis ellipsoidea*", a unicellular green alga that we use, can be grown in raceway ponds with total 33 kL working volume. However, the productivity of the oil always reduces during summertime and wintertime.

To cut down the production cost of biofuels, we need to achieve high productivity throughout the year. Choice of suitable production sites is one solution, but choice of strains suitable for culture in summer or winter could be the other answer. Thus searching and screening of microalgae which are appropriate for low or high temperature have been carried out.

Keeping the culture pH at very low level or at very high level plays very important role to reduce the risk of contamination by other microorganisms and zoo planktons. Therefore, we decided that pH is a second important selection pressure for screening microalgae.

口頭発表
Oral Presentation

Human health risk assessment and potential harm to natural habitats associated with large-scale algae cultivation

Misako Kato

Graduate School of Humanities and Sciences, Ochanomizu University

In a recent boom on researches on algal biofuels, those on artificial pond ecology and risk assessments are rare. This is one of the reasons why we carried out a risk assessment of human health and natural habitats associated with large-scale algae cultivation. The objective of our study is to provide experimental data for use in a comprehensive risk assessment of algae-based biofuel production systems.

We evaluated possible risk of *Pseudochoricystis ellipsoidea* cells to human health based on the oral acute toxicity test in rodents. We also examined the effect of *P. ellipsoidea* on plant growth, rice (*Oryza sativa*) and *Polygonum*. There was no indication of the harm of *P. ellipsoidea* to animals and plants.

The survival and growth of *P. ellipsoidea* cells were examined in water samples collected from various environments. It was demonstrated that the existence of nitrate was the prerequisite for *P. ellipsoidea* to grow in the water samples.

Finally, the susceptibility of *P. ellipsoidea* to herbicides was examined to identify agents capable of controling *P. ellipsoidea* in growing crop plants. The alga was susceptible to some but not all the herbicides.

口頭発表
Oral Presentation

Breeding better “energy algae”

Shigeaki Harayama

Department of Biological Science, Faculty of Science and Engineering, Chuo University

To achieve the commercialization of the microalgal fuel, the Agriculture, Forestry and Fisheries Research Council (AFFRC) started, in 2010, a comprehensive study to optimize the entire processes of the microalgal fuel production. This study is being undertaken in collaboration with Chubu University, Chuo University, DENSO Corporation, Kyoto University, Micro Algae Corporation, Ochanomizu University, Toyota Central R&D Labs., TOYOTA Motor Corporation Inc., and Saga University (alphabetic order). The goal of this project is to reduce significantly the production cost of the algal fuel, and the project includes the researches on (a) low cost cultivation methods, (b) low cost oil production methods from algal biomass, and (c) the production of value-added products from oil-extracted microalgal cells. The project also investigates (d) the environmental impact of mass-cultivation of microalgae in an open pond. In this joint research project, Chuo University is aiming at to improve genetically a microalga named “*Pseudochoricystis ellipsoidea*” for low-cost oil production.

The key traits to be improved in this alga include growth rate, oil-productivity, cell wall thickness, sedimentability, etc. It is known that the overall photosynthetic efficiency of an algal population could be improved by reducing the antenna size of the light-harvesting chlorophyll. Thus, we isolated several mutants possessing reduced amounts of chlorophyll. One of the mutants was tested for its biomass productivity in a large-scale open race-way pond, and the mutant was proven to be more productive than the wild-type strain.

To isolate mutants exhibiting higher productivity of triglycerides, a library of mutagenized cells was generated by treating with 1-methyl-3-nitro-1-nitrosoguanidine, and mutants expressing higher amounts of triglycerides were enriched with fluorescence activated cell sorting (FACS). The enrichment was repeated several times, and finally, we could isolate mutants expressing higher amounts of triglycerides than the wild-type strains.

Another type of mutants that we isolated showed altered permeability of the cell to organic solvents. From the wild-type cell of *P. ellipsoidea*, triglycerides could only be extracted after an acid pretreatment, however, from the mutant strains, triglycerides could be isolated without the acid pretreatment.

口頭発表
Oral Presentation

Toward Understanding Molecular and Cellular Mechanisms of Lipid Synthesis and Accumulation in Microalgae and Implications for Algal Biofuels Production

Qiang Hu

Laboratory for Algae Research and Biotechnology
Department of Applied Sciences and Mathematics
Arizona State University Polytechnic Campus
7001 E. Williams Field Road, Mesa, AZ 85212 (huqiang@asu.edu)

Many microalgae have the ability to synthesize and accumulate large amounts of storage neutral lipid mainly in a form of triacylglycerol (TAG) under stress (e.g., nutrient depletion, excess light). However, the pathways and regulatory mechanisms for TAG formation are poorly understood. Lipid bodies (LB) rich in TAG are subcellular organelles in microalgae. LB are limited in number and size when cells grow vigorously and are reproductive, but the number and size of LB may increase dramatically under stress. Although this phenomenon has been often observed, little is known about the formation and function of LB, especially under stress conditions. In this study, multiple pathways for TAG synthesis and the biogenesis and function of LB in *Chlamydomonas reinhardtii* were investigated using a systems biology approach. Several lines of evidence, including that obtained from confocal fluorescence and electron microscopy, and functional genomic, proteomic and lipidomic analyses of intact cells and isolated LB, suggest that phospholipid:diacylglycerol acyltransferase is to a large extent responsible for TAG synthesis under favorable culture conditions, whereas the Kennedy pathway plays a more important role in TAG formation under stress. The majority of LB are derived from and physically connected with the chloroplast. The intimate association of some LB with endoplasmic reticulum (ER) indicates that ER may be another route of LB formation. Our preliminary results indicate that LB are complex, dynamic organelles that interact actively with chloroplast, endoplasmic reticulum, and mitochondria. Genetic manipulation of key genes/enzymes associated with LB and closely related organelles that are involved in lipid metabolism may enhance the synthesis and sequestration of TAG into LB and thus increase overall TAG production.

ポスターセッション
POSTER SESSION

No.	発表代表者	所属	タイトル
No 1	Hisato Ikegaya 池谷　仁里	京都大学大学院・人間・環境学研究科	Screening of oil-producing microalgae 新規油脂蓄積微細藻類の探索
No 2	Kenshiro Shimada 嶋田　研志郎	京都大学大学院・人間・環境学研究科	Taxonomical characterization of three unicellular green algae isolated from acidic hot springs –importance of algae collection– 藻類探索の有効性 ― 強酸性温泉から分離された新規単細胞緑藻3株の分類学的考察 ―
No 3	Jumpei Hayakawa 早川　淮平	Department of Biological Science, Faculty of Science and Engineering, Chuo University	A low-chlorophyll mutant of green microalga *Pseudochoricystis ellipsoidea* with improved biomass productivity
No 4	Yoko Ide 井出　曜子	Department of Biological Science, Faculty of Science and Engineering, Chuo University	Isolation of mutants of *Pseudochoricystis ellipsoidea* with increased oil production
No 5	Minoru Kurata 倉田　稔	DENSO CORPORATION, Research Laboratories	CO_2 Reduction and Carbon Neutral Material Production by Microalgae
No 6	Satoko Komatsu 小松　さと子	DENSO CORPORATION, Research Laboratories	Development of cultivation process of *Pseudochoricystis ellipsoidea* in open-type ponds
No 7	Kanjana Khunathai クナタイ・カンジャナ	Department of Chemistry and Applied Chemistry, Saga University	Utilization of residual waste of oil-extracted microalgae for the recovery of noble metals
No 8	Izumi Matsuwaki 松脇　いずみ	お茶の水女子大学大学院・ライフサイエンス	Environmental impact assessment *of Pseudochorisistis ellipsoidea* by the model experiment system モデル実験系を用いたシュードコリシスティスの環境影響評価
No 9	Naohiro Goto 後藤　尚弘	Toyohashi University of Technology	Greenhouse Gas Balance on Life Cycle of Biodiesel: A Case of Palm Biodiesel Production in Indonesia
No 10	Kenichiro Inoue 井上　研一郎	独立行政法人土木研究所	Small algae culture by treated wastewater of sewage 下水処理水による微細藻類の培養
No 11	Osamu Miyashita 宮下　修	ジェイ・フェニックス・リサーチ株式会社	Critical factors for successful development and commercialization of algal biofuels in Japan: from the point of view of a financial analyst 証券アナリストから見た日本における藻類バイオ燃料産業の発展の成功ファクター

No.	発表代表者	所属	タイトル
No 12	Masaki Muto 武藤　正記	Institute of Engineering, Tokyo University of Agriculture & Technology	Development of Genetic Transformation for Marine Pennate Diatom *Fistulifera* sp. Strain JPCC DA0580 toward Biofuel Production
No 13	Michiko Nemoto 根本　理子	Institute of Engineering, Tokyo University of Agriculture & Technology	Genome-wide Analysis of Triglyceride Accumulation Mechanisms in Marine Pennate Diatom strain JPCC DA0580
No 14	Sousuke Imamura 今村　壮輔	Department of Biological Science, Faculty of Science and Engineering, Chuo University	Genetic analysis of "*Pseudochoricystis ellipsoidea*", an aliphatic hydrocarbon-producing green alga
No 15	Shigeaki Harayama 原山　重明	中央大学理工学部生命科学科	Genomic and transcriptome analyses of *Pseudochoricystis ellipsoidea* *Pseudochoricystis ellipsoidea*のゲノムおよびトランスクリプトーム解析
No 16	Yuya Yoshimitsu 吉満　勇也	㈱デンソー基礎研／中央大学	Molecular breeding of microalgae 微細藻類の育種
No 17	Yuji Yamaguchi 山口　裕司	マイクロアルジェコーポレーション㈱	Decontamination of radioactive Cesium by terrestrial blue green alga *Nostoc commune* 陸生藍藻イシクラゲ*Nostoc commune*による放射性セシウムの除染
No 18	Masaki Ota 大田　昌樹	Tohoku University	Effect of bubbling gas composition on photoautotrophic lipid production from a green alga *Chlorococcum littorale*
No 19	Motohiro Takenaka 竹中　元弘	Tohoku University	Supercritical fluid extraction of carotenoids from *Chlorococcum littorale* grown in photoautotrophic cultures
No 20	Jun Abe 阿部　淳	Faculty of Science, Japan Women's University	Construction of stable transformation system for a unicellular charophycean alga, *Closterium peracerosum-strigosum-littorale* complex
No 21	Tadaaki Simizu 清水　忠明	Department of Chemistry and Chemical Engineering, Niigata University	Drying of wet biomass by composting
No 22	Sayuri Ohta 太田　沙由理	新潟県農業総合研究所　基盤研究部	Recycle use of plant residues by the application of biological soil disinfestation 土壌還元消毒法を応用した野菜収穫残さの再利用
No 23	Yoji Kitajima 北島　洋二	Environmental Engineering and Bioengineering Group Kajima Technical Research Institute	Development of Novel Photo-Bioreactor for Photosynthetic Production of Hydrogen based on Light Compensation Hypothesis
No 24	Masanobu Kawachi 河地　正伸	National Institute for Environmental Studies	Algal Culture Collection in NIES

ポスター要旨

Poster abstract

新規油脂蓄積微細藻類の探索
Screening of oil-producing microalgae

○池谷仁里、嶋田研志郎、宮下英明　（京都大学大学院・人間・環境学研究科）

微細藻類を利用したバイオマス・エネルギー生産では、培養に掛かるコストの低減が重要な課題である。レースウェイなどの野外粗培養法は、チューブ型培養法に比べ生産コストを抑えることができる。しかし、その一方で空気中から原生動物や目的とは異なる藻類が混入して著しく生産効率が低下することが問題である。その対策として、混入生物の生育が困難な培養条件下において優勢かつ良好に生育する微細藻類を用いる方法が挙げられる。その例として、*Arthrospilla* sp.（スピルリナ）や“*Pseudochoricystis ellipsoidea*”の野外培養では、培地のpHをアルカリ性あるいは酸性に維持することによって混入生物の影響を低減させ、目的の藻類の優勢を維持している。本研究では、野外粗培養に適した特性をもつ油脂蓄積微細藻類の探索を目的に、pH 3およびpH 10の培地において、それぞれ35℃以上あるいは15℃以下で生育できる微細藻類を収集・分離を行った。その結果、日本各地の酸性・アルカリ性温泉や河川・湖沼から、単細胞緑藻を中心に530株を得た。更に、再現実験を行い、pH 3/35℃で88株、pH 10/35℃で58株、pH 3/15℃で30株、pH 10/15℃で97株がスクリーニングされた。これらの株の中には、蛍光染色法によって細胞内に相当量の油脂を蓄積するものも確認された。本研究により、野外粗培養で有用な株のスクリーニング方法が確立され、その成果は日本あるいは世界各地の気候に応じた微細藻類バイオマス生産を可能にする藻類資源の確保に繋がるものと考えられる。

藻類探索の有効性 – 強酸性温泉から分離された新規単細胞緑藻 3 株の分類学的考察 – Taxonomical characterization of three unicellular green algae isolated from acidic hot springs –importance of algae collection–

嶋田研志郎○[1]、藏野憲秀[2]、宮下英明[1]（[1]京都大学大学院・人間・環境学研究科、[2]デンソー(株)）

レースウェイ培養系など野外における微細藻類バイオマス生産では、温度管理が難しいため生産効率を維持する方法として、生育温度範囲の広い広域温度耐性株、あるいは、夏季の高温や冬期の低温にそれぞれ適した高温耐性株、低温耐性株を準備する必要がある。国内においても夏季の高温期には培養液温度が 40℃近くに達し、日本以南では特に高温耐性株の確保が不可欠である。高温環境の典型である温泉の微細藻類として、シアノバクテリア、紅藻類や珪藻類が主に知られている。中でも強酸性温泉（pH3 以下）には一部の紅藻類や珪藻が優勢し、緑藻類については極僅かの種の存在が知られているのみであった。一方で、温泉から分離された単細胞緑藻"*Pseudochoricystis ellipsoidea*" が強酸性に生育至適 pH をもつほか、近年の微生物群集構造解析から、強酸性温泉には新規の緑藻類が潜在していることが示されている(Ferris ら 2005)。本研究では、高温耐性藻類探索の過程において別府温泉および小松地獄の pH3 以下の環境から分離され、酸性培地中において生育した単細胞緑藻 3 株について、光学・電子顕微鏡観察および分子系統解析など分類学的検討を行った。その結果、いずれも緑色植物門 *Trebouxia* 藻綱の *Watanabea* クレードに帰属する 2 新属 2 新種であることがわかった。このことは、温泉環境にはまだ知られていない未知微細藻類が多数潜在しており、バイオマス生産効率の向上・維持には、これらの未知生物資源からの有用耐性株の探索が有効であることを示している。

A low-chlorophyll mutant of green microalga *Pseudochoricystis ellipsoidea* with improved biomass productivity

○Jumpei Hayakawa and Shigeaki Harayama
Department of Biological Sciences, Faculty of Science and Engineering, Chuo University

Microalgae-based biofuel production has many advantages over the biofuel production from land plants including much faster growing rate and much more energy productivity per area. Still, the production cost is a major barrier to utilize the algal biomass as a commercially viable fuel feedstock, and efforts to reduce capital and operating costs and to improve algae fuel productivity are required. Algal biomass productivity is directly dependent on the light-energy-capture efficiency of photosynthesis. When an algal culture reaches high density, light penetration is limited by excessive chlorophyll content of the cells. In order to improve an average productivity of cells in such culture, we isolated low-chlorophyll mutants of *P. ellipsoidea* after chemical mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine. The photosynthetic O_2 productivities per dry weight in dense cultures of the low-chlorophyll mutants were higher than that in the wild-type strain.

Isolation of mutants of *Pseudochoricystis ellipsoidea* with increased oil production

○Yoko Ide, Mika Sakamoto, Jumpei Hayakawa, Sousuke Imamura, and Shigeaki Harayama

Department of Biological Sciences, Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan.

Biodiesel production from microalgae is a promising approach to break the current dependence on fossil fuels, and the development of low-cost production system has attracted great interest. *Pseudochoricystis ellipsoidea* (MBIC11204) is a unicellular photosynthetic microorganism that can accumulate large amount of oils in cells under stressed conditions such as nitrogen deficiency. We aimed to isolate mutants with enhanced oil production for the advanced utilization of *P. ellipsoidea*. The oil vesicles in cells are stainable with fluorescent dyes such as Nile Red and BODIPY, and the fluorescent intensity is a good indicator of oil accumulation in living cells. For rapid screening of mutants with increased oil accumulation, flow cytometory analysis and cell sorting were performed using the fluorescent activated cell sorting (FACS) system. *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine-mutagenized *P. ellipsoidea* cells were cultivated in liquid media under several conditions and stained with BODIPY, then the cells with increased fluorescent emisson compared to those of the wild-type cells were selected by FACS. Individual colonies were isolated and the mutants with increased oil accumulation were finally selected, and further characterized.

CO_2 Reduction and Carbon Neutral Material Production by Microalgae

○Minoru Kurata and Kinya Atsumi
DENSO CORPORATION, Research Laboratories (minoru_kurata@denso.co.jp)

DENSO is developing a technology to capture CO_2 emissions from the industrial power plants by growing microalgae in open ponds. We also produce biodiesel fuels from the microalgae aiming to solve global warming and energy issues simultaneously and then to contribute to society.

A unicellular green alga, *Pseudochoricystis ellipsoidea*, patented by DENSO, grows quickly and has the ability to accumulate oil inside the cells up to about 30% of dry weight. DENSO is also trying to isolate several kinds of oil rich microalgae which can be grown at the temperature range unsuitable for *P. ellipsoidea.* It was possible to cultivate *P. ellipsoidea* at a pilot scale open ponds in our Zenmyo Plant. This alga could absorb CO_2 emission from 5 MW cogeneration power plants in Zenmyo. We could recycle the effluent of wastewater treatment facility as a growth medium, and could also reuse the waste heat generated by the cogeneration plant to dry the harvested cells. Highly oil-containing cells obtained in the Zenmyo facility could be directly used as a solid fuel, because the combustion heat of the cells was almost the same as coal. The extracted oil component could be converted into a biodiesel fuels through a conventional method. The residue of oil extraction is rich in essential amino acids, thus the utilization of it as a feeding stuff for cattle is one of the solutions for waste reduction. Noble metals adsorbent and/or cosmetic materials may be the other potential usage for oil extracted residues.

Development of cultivation process of *Pseudochoricystis ellipsoidea* in open-type ponds

○ **Satoko Komatsu**

DENSO CORPORATION, Research Laboratories (SATOKO_KOMATSU@denso.co.jp)

A unicellular green alga, *Pseudochoricystis ellipsoidea* has the ability to accumulate oil inside the cell under nitrogen starvation conditions and the oil component can be used as biofuels. This alga could grow under strong acidic conditions. We then expected that the risk of contamination by other algae and protozoa in a large-scale outdoor cultivation of *P. ellipsoidea* could be minimized when this alga is grown under a low pH. To prove it, *P. ellipsoidea* was continuously grown in open raceway ponds and microbial communities established in the open ponds were investigated with denaturing gradient gel electrophoresis (DGGE) analysis. The experiments were conducted using dual raceway ponds in our facility. In the first pond, cells of *P. ellipsoidea* were grown under nitrogen-sufficient conditions, and used as a seed culture for the second pond. In the second pond, the cells were cultivated in the form of a sequential batch culture by replacing half the culture volume with nitrogen-free medium to achieve the nitrogen starvation conditions. With this protocol, continuous cultivation was possible for one month. The microflora of the culture broth was analyzed by PCR-DGGE method, and it was found that significant contamination of microorganism was not observed during the long-term cultivation. This result thus demonstrated that the mass cultivation of this alga in open raceway ponds is possible.

Utilization of residual waste of oil-extracted microalgae for the recovery of noble metals

○Kanjana Khunathai[1], Katsutoshi Inoue[1], Hidetka Kawakita[1], Keisuke Ohto[1], Minoru Kurata[2], Hisaya Kato and Kinya Atsumi[2]

[1]Department of Chemistry and Applied Chemistry, Saga University, Honjo-1, Honjo, Saga 840-8502
[2]Research Laboratories, DENSO CORPORATION, Minamiyama 500-1, Komenoki, Nisshin, Aichi 470-0111

Recently, microalgae have been proposed as one of the new renewable resources for biofuel production due to rapidly increasing fuel prices, environmental, and resource concerns. In this current study, we have attempted to use the residual biomass waste generated from the extraction process of biofuel as a raw material to prepare the adsorption materials; which have been designed for the selective separation of noble precious metals. Crosslinked microalgae were prepared by crosslinking-condensation using concentrated sulfuric acid. Functional group-modified microalgae were prepared by immobilizing dithiooxamide and polyethyleneimine to chlorinated product of microalgal residue. Crosslinked microalgae exhibited high selectivity for Au(III) over other precious and base metal ions. From an isotherm experiment, the maximum adsorption capacity was evaluated to be >3 mol/kg for Au(III) which was many times higher than that of the original microalgal residue. SEM and X-ray diffraction confirmed the formation of metallic gold particles, suggesting the occurrence of a redox reaction between surface functional groups and Au(III). Dithiooxamide- and polyethyleneimine-immobilized microalgae exhibited high selectivity for Pd(II) and Pt(IV) adsorption irrespective of base metal ions in HCl medium. From the adsorption isotherms, these prepared adsorbents were found to have remarkably high adsorption capacity for Pd(II)>Pt(IV) via purely ionic interaction between anionic chloro complexes of metals and immobilized functional groups. The results observed in this work are promising for the practical use of microalgal residues to prepare the functional materials for the separation and recovery of noble metals.

モデル実験系を用いたシュードコリシスティスの環境影響評価

Environmental impact assessment of *Pseudochorisistis ellipsoidea* by the model experiment system

○松脇いずみ[1]、原山重明[2]、加藤美砂子[1]

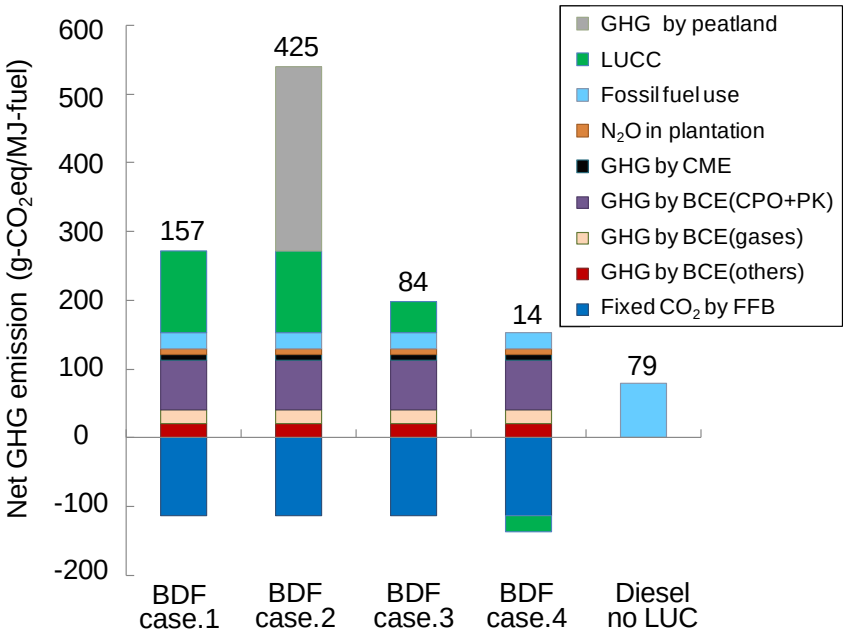
[1]お茶の水大・院・ライフサイエンス、[2]中央大・生命科学

シュードコリシスティスを屋外で大量に培養した場合、周辺環境に流出することが想定される。シュードコリシスティスが環境に与える影響を考えるために、河川や池から採取した水にシュードコリシスティスを加えて生残性試験を行った。その結果、採取した水に含まれる硝酸態窒素濃度が 2mg/L 以上の場合は、シュードコリシスティスは増殖した。硝酸態窒素濃度が検出限界以下の場合は、ほとんど増殖しなかった。さらに、水田から２週間ごとに水を採取し、同様の実験を行った。水を採取する時期により増殖傾向は異なるが、硝酸態窒素の有無が増殖に大きな影響を及ぼす点は先の結果と同じであった。フィルター濾過処理を行わず、捕食者等が混在する水を用いた場合、明らかに増殖は抑制された。また、シュードコリシスティスがイネの成長に及ぼす影響を調べるため、水耕栽培のイネ（*Oryza sativa* L. cv.nipponbare）にシュードコリシスティスを共存させた。シュードコリシスティスは、イネの成長に影響を与えず、ストレス応答の誘導も認められなかった。

Greenhouse Gas Balance on Life Cycle of Biodiesel: A Case of Palm Biodiesel Production in Indonesia

Hirotsugu Kamahara and ○Naohiro Goto
Department of Environmental and Life Sciences, Toyohashi University of Technology
Tempaku, Toyohashi, 441-8580, Japan
goto@ens.tut.ac.jp

This study evaluated the greenhouse gas (GHG) balance on the life cycle of biodiesel fuel (BDF). As the case study, importing BDF derived from palm oil of Indonesia to Japan was considered. Net GHG emission was calculated by considering biomass carbon balance, fossil energy use and land use change. Land use change to palm plantation from natural rainforest, natural rainforest on peatland, logged over natural rainforest and degraded land were considered for evaluating that GHG emission. The result showed that net GHG emission in case of land use change from degraded land was the lowest emission in all case of BDF and it had 18% of GHG emission in case of diesel use. Furthermore, when carbon sequestration was counted as negative for net GHG emission in this case, net GHG emission was estimated as negative value. On the other hand, the case of diesel use has disadvantage by no land use change. In this reason, the land use change from degraded land to afforestation of acacia was considered, and that result of net GHG emission was lower than all cases of BDF.



Net GHG emission on each case (BDF case of land use change to plantation from 1:natural rainforest, 2:natural rainforest on peatland, 3:logged over forest, 4:degraded land.)

下水処理水による微細藻類の培養
Small algae culture by treated wastewater of sewage

○井上研一郎、桜井健介、岡本誠一郎
独立行政法人 土木研究所

微細藻類のバイオ燃料の実用化が期待されている。また、微細藻類の培養に下水を用いた場合、下水中に含まれる栄養塩の除去、低減が可能となることから、下水の高度処理技術としても注目されている。

このような背景から下水二次処理水(最終沈殿池流入水)を用いて、半連続式の培養装置(容量: 2 L)により微細藻類を培養した。外部からの微細藻類の株や栄養塩を添加することなく、微細藻類の培養が可能であることが示された。さらに、培養された微細藻類は、*Scenedesmus* 科が 90%以上を占め、単一科が優占していることが示された。また、この培養により処理水中の栄養塩(リン、窒素)濃度が低減した。本実験で用いた下水二次処理水においては、その性状からリンが微細藻類の増殖を制限しているものと考えられた。微細藻類のバイオ燃料化、さらに微細藻類の培養により下水処理を高度化することが有望であるものと考えられた。

証券アナリストから見た日本における藻類バイオ燃料産業の発展の成功ファクター

宮下　修
ジェイ・フェニックス・リサーチ株式会社

藻類バイオ燃料産業の発展には、数多くの基礎・要素技術の開発と、事業化に向けた産官学の連携、各種の規制整備を含めた様々な課題、ハードルが存在している。それらの克服には、関係者にとって、総合的な判断が可能となるための知識・知見・経験の体系化、構造化を進めることが極めて重要である。最大のボトルネックは、１リットル１００円程度の燃料生産の実現に必要な低コスト大量培養技術の確立であるが、世界で実現にめどをつけている企業は皆無である。日本は、基礎研究では世界をリードし、クロレラ、スピルリナ、ドナリエラ、ユーグレナなど世界に先駆けて、大量培養したノウハウを持つ。こうした知的・経営的資源を活用し、戦略的な投資、産官学の連携を行なっていけば、藻類バイオ燃料産業において世界のトップに立つことは十分に可能であると考える。当社は、藻類産業創成コンソーシアム（理事長：筑波大学井上勲教授）において、平成 23 年度農山漁村６次産業化対策事業で「農山漁村における藻類バイオマスファームの事業化可能性調査」に関わっている。このプロジェクトでは、藻類培養の豊富な経験を持つ企業に加え、総合エネルギー、化学、化粧品、食品、エンジニアリング、水処理、など藻類産業発展に関連する日本を代表する企業が数十社参画している。当社は、証券アナリストとして、総合的、長期的な視点で、藻類産業発展の成功ファクターを分析し、産業複合的な動きを推進に貢献していきたいと考える。

Development of Genetic Transformation for Marine Pennate Diatom *Fistulifera* sp. Strain JPCC DA0580 toward Biofuel Production

○Masaki Muto[1,3], Yorikane Fukuda[1], Michiko Nemoto[1], Tomoko Yoshino[1], Mitsufumi Matsumoto[2,3], Tadashi Matsunaga[1] and Tsuyoshi Tanaka[1,3]

[1]Institute of Engineering, Tokyo University of Agriculture & Technology, [2]Biotechnology laboratory, Electric Power Development Co., Ltd, [3]JST, CREST

Marine pennate diatom *Fistulifera* sp. strain JPCC DA0580 has been newly identified as the highest triglyceride (60%) producer from a marine microalgae culture collection for biodiesel fuel (BDF) production. In order to improve the economics of BDF production, a genetic manipulation technique for strain JPCC DA0580 was examined by using microparticle bombardment. The transformation conditions for strain JPCC DA0580 were optimized using the green fluorescent protein gene (*gfp*) and the gene encoding neomycin phosphotransferase II (*nptII*). Efficient transformation using microparticle bombardments was conducted at 0.6 μm of tungsten particles under a pressure condition of 450 psi (transformation efficiency: $> 10^{-7}$). Furthermore, the endogenous promoters (*F-fcpB*, *H4*, and *rpoA*), selected from the whole genome data of strain JPCC DA0580 were found to be useful for obtaining transformants with high efficiency.

Genome-wide Analysis of Triglyceride Accumulation Mechanisms in Marine Pennate Diatom strain JPCC DA0580

○Michiko Nemoto[1], Yorikane Fukuda[1], Masaki Muto[1,3], Tomoko Yoshino[1], Mitsufumi Matsumoto[2,3], Tadashi Matsunaga[1] and Tsuyoshi Tanaka[1,3]

[1]Institute of Engineering, Tokyo University of Agriculture & Technology, [2]Biotechnology laboratory, Electric Power Development Co., Ltd, [3]JST, CREST

Marine microalgae with seawater-requirement for growth have several advantages in practical application for biofuel production due to low land costs and free seawater, and repressing contaminant microorganisms from preferentially growing. A marine pennate diatom, *Fistulifera* sp. strain JPCC DA0580 was identified as the highest neutral lipid-producer (The lipid content: 60%) among 1393 strains by screening a marine microalgal culture collection. In order to accelerate metabolic engineering toward the biodiesel production, the whole genome of strain JPCC DA0580 was sequenced. The comprehensive genome analysis provided better insight about the metabolic pathways in strain JPCC DA0580. Furthermore, a digital gene expression analysis was conducted based on the whole genome data. In the analysis, total RNA were extracted from the triglyceride accumulating and non-accumulating cells prepared by different culture condition and sequenced by Illumina Genome Analyzer. A number of genes up-regulated only in triglyceride accumulating cells might be important for triglyceride synthesis in this strain.

Genetic analysis of "*Pseudochoricystis ellipsoidea*", an aliphatic hydrocarbon-producing green alga

°Sousuke Imamura[1], Daisuke Hagiwara[1,2], Fumi Suzuki[1], Norihide Kurano[2], Shigeaki Harayama[1]

[1]Department of Biological Sciences, Faculty of Science and Engineering, Chuo University
[2]Research Laboratories, DENSO CORPORATION

Pseudochoricystis ellipsoidea is a unicellular green alga and has a unique ability to synthesize and accumulate intracellularly a significant amount of aliphatic hydrocarbons (1). The productivity of the hydrocarbons is increased under the nitrogen deprivation condition. To elucidate molecular mechanisms of the hydrocarbon production in this organism, the development of genetic methods including DNA transformation methods are important. Towards the goal, we constructed several plasmids in which neomycin phosphotransferase II-encoding G418-resistant gene (*nptII*) is flanked by *P. ellipsoidea*-derived promoter and terminator. These plasmids were introduced into *P. ellipsoidea* cells through particle-gun bombardment, and transformants were screened among G418-resistant cells by PCR amplification of plasmid-borne genes. Southern blot analysis demonstrated that the exogenous DNA was integrated into the genome of the transformants. Furthermore, the expression of *nptII* was confirmed at the transcript and protein levels by RT-PCR and immunoblot analyses, respectively. These results clearly indicated that a genetic transformation system was successfully established for *P. ellipsoidea* (2).

Based on this genetic transformation technique, we are now trying to improve the productivity of hydrocarbons by constitutive expression of transcription factors of which gene expression are induced under the nitrogen deprivation condition, and will discuss the strategy with recent results on the challenge.

(1) Satoh *et al*. (2010) *J. Jpn. Inst. Energ.*, **89**, 909–913.
(2) Imamura *et al*. *J. Gen. Appl. Microbiol.* in press.

Pseudochoricystis ellipsoidea のゲノムおよびトランスクリプトーム解析

原山　重明[1]、今村　壮輔[1]、藏野　憲秀[2]、近藤　伸二[3]、Todd Taylor[3]、鈴木 穣[4]
[1]中大生命科学、[2]デンソー、[3]理研メタシステム、[4]東大新領域

Pseudochoricystis ellipsoidea のゲノム解析を実施した。アセンブリングの結果、塩基配列は、118 の scaffolds に連結された。そして、これらの scaffold の長さを足し合わせると、49.6 Mb となった。

引き続き、RNA-seq 法による網羅的遺伝子発現解析を行った。RNA 配列を基に、intron および exon を決定した。intron 総数は 10 万以上、一つの遺伝子には、平均して、10 近くの intron が存在した。Intron skipping が高い頻度で観察されたが、その生物学的意味は現在のところ不明である。

Molecular breeding of microalgae（微細藻類の育種）

○吉満　勇也

㈱デンソー基礎研／中央大学

微細藻類を使ったエネルギー生産のコスト競争率は乏しく、エネルギー回収効率を高めた育種が必要である。そのため、遺伝子組み換え技術を利用した迅速な育種法の開発は急務である。

Pseudochoricystis ellipsoidea は、屋外開放系においての培養が可能な緑藻で、窒素欠乏時に油脂を蓄積する。この特性を持つ *P. ellipsoidea* をさらに改良するため、当グループでは薬剤を使って遺伝子突然変異を誘起させ、低クロロフィル突然変異体・高油脂突然変異体・細胞壁透過性突然変異体などの有用な形質を獲得した変異株を分離してきた。しかし、これらの遺伝子変異は、それぞれ独立した株の中に存在する。そこで、次のステップとして上記の優良形質を生んだ突然変異を一つの株（新しい宿主）に集積する。新しい機能をもたらす突然変異（gain of function）の場合、その突然変異遺伝子をクローン化し、新しい宿主に移せば良い。しかし、ある遺伝子が不活化されたために形質が改良されたような場合（loss of function)、その遺伝子を宿主内でピンポイントに不活化する工夫が必要である。その方法として、遺伝子の knockdown と knockout を考えている。前者としては、artificial micro RNA（amiRNA）を使って遺伝子発現やタンパク質合成を抑制する方法を用いる。後者としては、特定の塩基配列を認識するタンパク質とエンドヌクレアーゼとを組み合わせた Transcription activator-like effector nuclease (TALEN)という最新技術を使い、部位特異的な DNA 切断を試みる。

まず、モデル生物である *Chlamydomonas reinhardii* を使って実験系を確立させる。続いて、その技術を *P. ellipsoidea* に適用し、最終的には優良突然変異を統合させた株を作出する。

陸生藍藻イシクラゲ *Nostoc commune* による放射性セシウムの除染

Decontamination of radioactive Cesium by terrestrial blue green alga *Nostoc commune*

○山口裕司* ・ 櫻田　修** ・纐纈　守** ・ 佐々木秀明*** ・ 佐藤健二*** ・ 竹中裕行*

*マイクロアルジェコーポレーション㈱　・ **岐阜大学工学部 ・ ***いわき明星大学科学技術学部

東京電力福島第一原子力発電所の事故に伴い、福島県を中心に広範囲にわたり、放射性物質に汚染された。ヒマワリなどの植物による放射性セシウムの生物除染が検討されているが、これまでのところ、有効な植物は得られていない。

陸生藍藻のイシクラゲ *Nostoc commune* は強い放射線の中でも生存できることから[1)]、我々はイシクラゲによる表土の放射性物質除染の可能性を調べるために、イシクラゲのセシウムの吸収能について調べた。

安定同位元素セシウムを添加した培地で気生培養したところ、イシクラゲ中のセシウム濃度は添加量に比例して増加した。 さらに、福島県のいわき市と二本松市に自生しているイシクラゲを採取・洗浄し、放射線量を測定した結果、最大で 81,700Bq/kg の放射性セシウムが検出された。イシクラゲが自生していた表面から 5cm 以内の土壌の放射線量は 15m ほど離れたイシクラゲのいない土壌より約 4 割から 5 割減少していた。

以上の結果より、イシクラゲによる生物除染の可能性が示唆された。

1)　Potts M., Eur. J. Phycol.(1999).

Effect of bubbling gas composition on photoautotrophic lipid production from a green alga *Chlorococcum littorale*

○Masaki Ota, Motohiro Takenaka, Yoshiyuki Sato, Hiroshi Inomata
Research Center of Supercritical Fluid Technology, Tohoku University

Photoautotrophic fatty acid production of a highly CO_2-tolerant green alga *Chlorococcum littorale* was investigated in the presence of inorganic carbon and nitrate at 295 K and a light intensity of 170 μmol-photon m^{-2} s^{-1}. CO_2 concentration in the bubbling gas was adjusted by mixing pure gas components of CO_2 and N_2 to avoid photorespiration and β-oxidation of fatty acids under O_2 atmosphere conditions. Fatty acid content was found to be almost constant for the CO_2 concentrations ranging from 5% to 50% under nitrate-rich conditions corresponding to the logarithmic growth phase. After nitrate depletion, the fatty acid content drastically increased with a decrease in CO_2 concentration. HCO_3^-/CO_2 ratio in the culture media was estimated to be a controlling factor for fatty acid production after the nitrate limitation phase. For a CO_2 concentration of 5%, the fatty acid content was ca. 34 wt% at maximum, which is comparable with other land plant seed oils.

Supercritical fluid extraction of carotenoids from *Chlorococcum littorale* grown in photoautotrophic cultures

○Motohiro Takenaka, Hiromoto Watanabe, Masaki Ota, Masaru Watanabe,
Yoshiyuki Sato, Hiroshi Inomata
Research Center of Supercritical Fluid Technology, Tohoku University

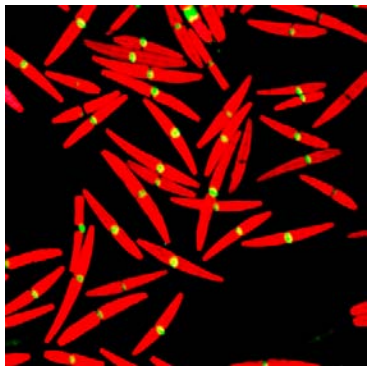
Carotenoid production from a highly CO_2 tolerant microalga *Chlorococcum littorale* grown in photoautotrophic cultures with supercritical fluid processing was studied. Increasing temperature, increasing light intensity and decreasing CO_2 and O_2 gas concentrations enhanced growth rate under nitrate-rich conditions. Carotenoid content was insensitive to temperature and gas composition, but was greatly promoted by light intensity in a logarithmic growth phase. It was found that the growth rate and carotenoid content preferred to the light intensities of ca. 120 μ mol-photon $\cdot$ m^{-2} s^{-1}. The supercritical CO_2 extractions with or without 10 mol% ethanol as co-solvent have been conducted for the alga sample. Extraction yield of carotenoids was 90% with 10 mol% ethanol at 333 K and 30 MPa and 180 min.

Construction of stable transformation system for a unicellular charophycean alga, *Closterium peracerosum-strigosum-littorale* complex

○ Jun Abe[1], Sachie Hori[2], Hiroyuki Sekimoto[1]

[1] Faculty of Science, Japan Women's University, Japan
[2] Graduate school of science, Japan Woman's University, Japan

Although charophycean algae form a relevant monophyly with embryophytes and hence occupy a fundamental place in the development of Streptophyta, no tools for genetic transformation in these organisms have been developed. We therefore construct a stable nuclear transformation system for the unicellular charophycean alga, *Closterium peracerosum-strigosum-littorale* complex (*C. psl* complex). When a vector, pSA106, containing the dominant selectable marker *ble* (phleomycin-resistant) gene and a reporter *cgfp* (*Chlamydomonas*-adapted GFP) gene was introduced into cells via particle bombardment, a total of 19 phleomycin-resistant cells were obtained in the presence of a low concentration of phleomycin. Six isogenic strains isolated using conditioned medium showed consecutive *cgfp* expression and long-term stability for phleomycin resistance. DNA analyses verified single or tandem/redundant integration of ~10 copies of pSA106 into the *C. psl* complex genome. We also constructed an over-expression vector, pSA1102, and then integrated a *CpPI* gene encoding minus-specific sex pheromone into pSA1102. Ectopic over-expression of *CpPI* and the pheromonal function were confirmed when the vector pSA1102_*CpPI* was introduced into the mt^+ cells. The present efficient transformation system for the *C. psl* complex should provide not only a basis for molecular investigation of *Closterium* but also an insight into important processes in early development and evolution of Streptophyta.



Closterium transformants obtained by introduction of *ble-cgfp* fusion gene.

Drying of wet biomass by composting

○Tadaaki Simizu[1*], Shoiti Iida[2], Heejoon Kim[1], Liuyun Li[1]

[1]Department of Chemistry and Chemical Engineering, Niigata University

[2]Graduate School of Science and Technology, Niigata University

*Corresponding author : tshimizu@eng.niigata-u.ac.jp

Waste mushroom culture (WMC) is an agricultural byproduct of mushroom production. The annual WMC production in Toukamachi City area, Niigata Prefecture, Japan, is more than 10,000 tons and WMC is expected to be used as a biomass fuel. Prior to use, however, moisture content of WMC must be reduced from about 55% in raw material to about 30 - 40% so that WMC can burn without supplementary fuel. In this work, we proposed drying of WMC by use of fermentation heat during composting as a low energy consumption and low cost drying method. In this study, WMC was dried by composting with feeding air under an aerobic condition with seed compost. Experiments of composting the mixed samples were conducted using a bench-scale fixed bed composter. In the experiments, temperature change, CO_2 formation rate, and water evaporation rate were measured. Addition of seed compost was found to be effective to enhance fermentation. A heat balance model was also established to predict the temperature and rate of water evaporation during composting.

土壌還元消毒法を応用した野菜収穫残さの再利用
Recycle use of plant residues by the application of biological soil disinfestation

○太田沙由理・古川勇一郎・前田征之・白鳥豊
新潟県農業総合研究所

食料自給率向上を目指した野菜の生産拡大に伴い収穫残さの発生量が増大している。収穫残さには肥料成分が多く含まれているものの、病原菌等の伝染源となる恐れがあるため資源化技術が未確立であり、現状では多くが焼却・埋却・放置され、環境汚染や鳥獣害の面で大きな問題となっている。そこで、土壌病害の抑止に効果がある土壌還元消毒法を応用し、新たな資源化技術を開発する。

真空パックを用いた密閉培養試験では、土壌還元消毒法と同様にトマト茎葉中の青枯病菌を死滅させることが可能性であった。青枯病菌の死滅には嫌気発酵に伴う有機酸の生成が関与しており、有機酸を多く蓄積させる手法として尿素の添加が有効であることを明らかにした。

ドラム缶を用いて嫌気処理を行ったトマト収穫残さについても、茎葉中から青枯病菌は分離されなかった。現在、有機酸の蓄積量、肥料成分を分析するとともに、資源化物を施用して葉菜類を栽培し、生育に及ぼす影響を調査している。

Development of Novel Photo-Bioreactor for Photosynthetic Production of Hydrogen based on Light Compensation Hypothesis

○Yoji KITAJIMA, Yoshiyuki UENO, Seiji OTSUKA, Masafumi GOTO

Environmental Engineering and Bioengineering Group

Kajima Technical Research Institute

19-1, Tobitakyu 2-Chome, Chofu-shi, Tokyo 182-0036

JAPAN

E-mail: kitajima-yoji@kajima.com

Microbial production of hydrogen by photosynthetic bacteria was investigated in order to develop a practical photo-bioreactor. The light compensation hypothesis suggests that the net production of hydrogen is the balance between gross hydrogen production and hydrogen "potential" utilized for microbial growth and/or converted to by-products other than hydrogen. A novel photo-bioreactor, which is capable of stably producing hydrogen regardless the sunlight conditions by adjusting the effective surface area, was designed based on the hypothesis. The data obtained by the experiments conducted on the photo-bioreactor with *Rhodobacter sphaeroides* strain RV and organic wastewater indicate that the overall efficiency of photosynthesis can be improved by the new design.

This work was supported by the New Energy and Industrial Technology Development Organization (NEDO).

Keywords: photo-bioreactor, hydrogen production, light compensation, *Rhodobacter sphaeroides*, photosynthetic bacteria

Algal Culture Collection in NIES

○Masanobu Kawachi, Mikihide Demura and Fumie Kasai
National Institute for Environmental Studies (NIES)
16-2 Onogawa, Tsukuba, 305-8506 Japan
E-mail: mcc@nies.go.jp, kawach9i@nies.go.jp

Recently, algae have attracted considerable attention as bioresources that produce valuable materials, economically as well as environmentally, such as biofuels. Furthermore, algae are known as important primary producers that carry out half of the global primary production making them crucial to the creation and regulation of the global environment. Algae are expected to be even more important in future research to create “low carbon societies”. And diversity of algal bioresources is essential to deal with these issues.

The Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection) was established as an “environmental study-oriented” culture collection in 1983. The NIES-Collection currently maintains more than 2,700 strains, among which ca. 2,200 strains are available to the public. These strains include, in addition to model organisms, evolutionarily and ecologically important organisms. They belong to more than 17 phyla that extend to prokaryotic cyanobacteria and, at least, 6 eukaryotic supergroups.

NIES-Collection is a public dedicated culture collection and have distributed strains for many purposes as well as received new strain depositions. Constant and smooth connection exists in between the algal research projects in NIES and the NIES-Collection. Bilateral benefits between project and culture collection will be introduced by two recent projects concerning marine picophytoplankton diversity and hydrocarbon producing algae.

農林水産技術会議事務局
TEL:03-3502-8111
http://www.s.affrc.go.jp/
中央大学研究開発機構
TEL:03-3817-1603
k-shien@tamajs.chuo-u.ac.jp
http://www.chuo-u.ac.jp