PHYCONET Annual Conference 2015

Wednesday 26th August at the Novotel London West (1 Shortlands, London W6 8DR)

Programme

9.45: Registration

Session 1: PHYCONET (Chair: Michele Stanley - PHYCONET Co-Director, Scottish Association for Marine Science)

10.15: Saul Purton (PHYCONET Director)
PHYCONET summary

10.30: Tracey Beacham (Plymouth Marine Laboratory)
ALGEBRA: ALGal Environmental and Biotechnological risk Assessment

11.00: Carole Llewellyn (Swansea University)
Getting the light right

11.30: Tom Bibby (University of Southampton)
Realising the biotechnological potential of marine microalgae

12.00: Lunch

Session 2: Early Career Researcher session (Chair: Olga Sayanova - Rothamsted Research)

13.30: Elahe Radmaneshfar (University of Aberdeen)
Mathematical modelling of fatty acid biosynthesis in microalgae

13.50: Maria Huete-Ortega (University of Sheffield)
Linkage between photosynthesis and nitrogen metabolism on the accumulation of lipids in microalgae

14.10: Sara Abalde-Cela (University of Cambridge)
High-throughput detection of ethanol-producing cyanobacteria in a microdroplet platform

14.30: Thea Coward (Swansea University)
Manufacturing with light: utilising light-emitting diodes for controllable and reproducible production of high-value products from microalgae

14.50: Ebenezer Ojo (University College London)
Scale-down technologies for rapid and scalable microalgae bioprocessing

15.10: Coffee break

Session 3: Towards commercialisation (Chair: Brenda Parker - University College London)

15.45: Vítor Verdelho (A4F, EABA President)
Microalgae production technologies and extreme microalgae

16.15: Michael Graz & Almero Barnard (Neem Biotech Ltd)
Considerations for downstream processing of microalgae and thoughts on commercialisation

16.45: Emmanouil Flemetakis (AlgaeCom project Co-ordinator, Agricultural University of Athens)
ALGAECOM: mining and optimizing microalgae biomass for novel cosmeceuticals

17.15: Pattanathu Rahman (TeeGene Biotech Ltd)
Biosurfactants and Bioemulsifiers from microalgae

17.45: Coffee and announcement of prizes

Presentation abstracts

ALGEBRA: ALGal Environmental and Biotechnological risk Assessment (Tracey Beacham, PML)

The naturally high metabolic diversity of microalgae coupled with high growth rates and the ability to grow in seawater (and in industrial waste water streams or grey water), removes a significant reliance on the worlds' increasingly valuable fresh water supplies. The industrial biotechnology sector has been quick to respond to such opportunities and in recent years has seen a rise in interest in commercial production of microalgal biomass for its associated metabolic products and services. Whilst many key species have become successfully established as suitable for mass culture, the advent of the genomic era has heralded a new dawn in microalgae potential by allowing the combination and selection of key physiological characteristics with modified metabolic activities. Put simply, genetically modified (GM) algae have the potential to revolutionise food, feed, fuel and pharmaceutical production. However, commercialisation of such microalgae will require the culturing of GM microbes on an unprecedented scale. At present there is a fundamental lack of information and assessment tools available to researchers, industrial developers or regulators on the risks associated with their large scale propagation. This project seeks to identify characterise assess and scrutinise the factors that will need to be considered in order to progress GM microalgae scale up in both closed and open systems.

Getting the light right (Carole Llewellyn, Swansea University)

The intensity and spectral distribution of light are crucial factors in determining the overall productivity and production and optimisation of useful metabolites in algae. Here I will present aspects on using both ends of the light spectrum, ultraviolet (UV) and Far Red Light (FRL). Some algae are able to protect against the damaging effects of UV by producing sunscreens and these have potential use in the cosmetics/personal care industry. In my talk I will present some experimental results from previous trials. At the other end of the light spectrum, FRL has been shown to be linked to a new chlorophyll with absorption in the far red (720nm) potentially important in increasing productivity in dense cultures. Here I will present an introduction and plans for the PHYCONET Proof of Concept funded research.

Realising the biotechnological potential of marine microalgae (Tom Bibby, University of Southampton)

Marine photosynthetic microbes (microalgae) have great potential in biotechnology. They have huge genetic diversity and naturally make an array of metabolites that are precursors in high-value products such as fuels and pharmaceuticals. They do not compete with agriculture for land or fresh water and can be used to reduce industrial carbon emissions. In order to realize this potential much work needs to be done to overcome the challenges of developing genetic tools required to increase the yield and diversity of products synthesized by poorly characterized (non-model) microalgal systems.

Here we report on a high-throughput pipeline merging random mutagenesis and single-cell sorting with next-generation sequencing technologies to select marine algal strains with improved photosynthetic efficiency for growth. Further, we report on a recently initiated BBSRC-funded PHYCONET award in partnership with Algenuity through which we are developing a highly efficient genetic transformation protocol that enables targeted genetic modification, promoter identification and heterologous gene expression in *Dunaliella*. Together, these 'random' and 'targeted' approaches represent the fundamental technologies necessary to begin to realize the biotechnological of potential poorly characterized marine microalgae.

Mathematical modelling of fatty acid biosynthesis in microalgae (Elahe Radmaneshfar, University of Aberdeen)

Essential fatty acids are those that are good for health but cannot be synthesized by mammals (including humans).

The main source of these fatty acids (i.e. *omega-3*) are fish, but overfishing causes severe ecological problems. One great source of these essential fatty acids are algae, however producing fatty acids from these micro-organisms in industrial scale requires detailed understanding of fatty acid biosynthesis in general. In this talk I will present the very first mathematical model which addresses the combinatorial explosion of pathways to synthesis fatty acids.

This combinatorial complexity arises from the unspecificity of elongase and desaturase enzymes. Our stochastic model predicts the distribution of different fatty acids over time for given external conditions.
It also predicts the abundance of the final product when the ratio of elongase vs desaturase can be controlled. Furthermore, it reveals the influence of rate of delta and omega desaturase on the fatty acid distribution pattern.

Linkage between photosynthesis and nitrogen metabolism on the accumulation of lipids in microalgae

Maria Huete-Ortega[1], M.P. Johnson[2], D.J. Gilmour[2], K. Okurowska[1] & S. Vaidyanathan[1]

[1]Department of Chemical and Biological Engineering, University of Sheffield; [2]Department of Molecular Biology and Biotechnology, University of Sheffield

Photosynthetic microalgae have a great potential for biofuel production. In microalgae, both growth and lipid production are a physiological trade-off, resulting from balancing the resource and energy allocation within the cells amongst the anabolic and catabolic pathways. In this sense, lipid production in microalgae has been suggested to act as a sink of the chemical energy and reductant power resulting from photosynthesis when cell growth is arrested. Therefore, in order to better understand the lipid production pathway, the linkage between both photosynthesis and nitrogen assimilation was investigated in the present research. Two taxonomically different species (*Nannochloropsis oceanica* and *Phaeodactylum tricornutum)* of microalgae were grown in batch cultures at high light intensity and under different nitrogen sources (Nitrate, Nitrogen free and Ammonia). Changes in the growth rate, biochemical composition and photophysiology by Chlorophyll *a* fluorescence methodology were examined to establish trade-offs in their physiological response towards biofuel production. Both species presented a significant decrease in growth rate in the Nitrogen free treatment with respect to the Nitrate and Ammonia, matching the decrease in Chlorophyll *a* and protein contents. Conversely, total fatty acids were higher in the Nitrogen free treatment, but similar to the content obtained in the Ammonia treatment. In both species, Chlorophyll a fluorescent measurements indicated the photodamage of the Photosystem II in the Nitrogen free treatment (significant lower maximum quantum yields and photosynthetic efficiencies), while marked differences in the amount of phototochemical (qP) and non-photochemical quenching (NPQ) were found between them. The role of the NPQ on lipid production was further investigated.

High-throughput detection of ethanol-producing cyanobacteria in a microdroplet platform

Sara Abalde-Cela[1], Anna Gould[1, 2], Xin Liu[1, 3], Elena Kazamia[1], Alison G. Smith[4], Chris Abell[1]

[1]Department of Chemistry, University of Cambridge; [2]Institute of Process Engineering (Zurich, Switzerland); [3]Sphere Fluidics,Cambridge; [4]Department of Plant Sciences, University of Cambridge

Ethanol production by microorganisms is increasingly an important renewable energy source. Most processes involve fermentation of sugars from plant feedstock, but increasingly there is interest in direct ethanol production by photosynthetic organisms. However, several challenges remain to optimise the system, and to facilitate this, high-throughput screening techniques for the detection of ethanol are needed. In this paper, we describe a method for the quantitative detection of ethanol in a microdroplets-based platform that can be used for screening cyanobacterial strains to identify those with the highest ethanol productivity levels.[1] The indirect detection of ethanol by enzymatic assay was first optimized both in bulk and in microdroplets. In parallel, we demonstrated the encapsulation of engineered ethanol-producing cyanobacteria in microdroplets, and their growth dynamics in microdroplet reservoirs. The combination of modular microdroplet operations including droplet generation for cyanobacteria encapsulation, droplet re-injection and pico-injection, and laser-induced fluorescence, allowed us to establish a new platform able to screen genetically-engineered strains of cyanobacteria with different levels of ethanol production.

Manufacturing with light: utilising light emitting-diodes for controllable and reproducible production of high-value products from microalgae

Thea Coward[1], Claudio Fuentes Grünewald[1], Darren L. Oatley-Radcliffe[2] , Robert W. Lovitt[1]

[1]Centre For Complex Fluids Processing (CCFP), Swansea University; [2]Energy Safety Research Institute (ESRI), Swansea University

The demand for sustainable high-value proteins, pigments, carbohydrates, and lipids derived from microalgae is increasing rapidly. In order for this to be realised, production needs to be reliable and with robust methods, which will be required to meet strict pharmaceutical standards. Photobioreactors provide an important role in the production control to ensure the quality standards required for the production of active pharmaceutical ingredients are met, as they allow for the common biotic (e.g. pathogen contamination and competition with other microorganisms) and abiotic (e.g. temperature, gases, pH, and nutrients) bottlenecks in microalgal growth to be greatly reduced or even removed. The quality and quantity of light required is paramount for the control of growth and product formation, therefore light is critical processing parameter, that needs to reliable, controlled, and reproducible to gain a consistent products. However, this is not possible when utilizing natural sunlight for cultivation due to fluctuations of light intensity, quality, and photoperiod. Light-emitting diodes (LEDs) are a relatively cheap and efficient modern lighting source that give the algae physiologist and process engineer a series of powerful tools to manipulate the production system for enhancing product formation, while simultaneously eliminating variability associated with natural light. LEDs are the only light source that truly facilitates tailored lighting - which allows the full control and manipulation of the spectral quality, intensity, flashing light effect, and the photoperiod of light, which have proven to be particularly important for algal growth,

metabolism, and product formation manipulate the production system for enhancing product formation, while eliminating variability associated with natural light. The effect of specific wavelength on the growth and product formation of three commercially important species (*Haematococcus*, *Porphyridium*, and *Nannochloropsis*) has been conducted at small scale. This data has then been used to create a 1000 L scale PBR with Internally mounted LED light panels. LED lighting may therefore provide the step-change required in PBR design to produce a controllable, adaptable, reproducible, robust system for production of high value products.

Scale-down technologies for rapid and scalable microalgae bioprocessing

Ebenezer Ojo, Hadiza Auta, Frank Baganz & Gary J. Lye, University College London

Investigation of promising strains of microalgae for biopharmaceutics, therapeutics, and other high-value chemicals are on the increase. The need for scale-down technologies has become essential for gaining early insights into characteristics of potential strains and the bioprocess development options. Of particular interests are the development and application of scale-down technologies such as a high-throughput platform for cultivation and ultra scale-down tools for downstream biomass and product recovery. This presentation features successful applications of a miniature photobioreactor using *Chlorella sorokiniana* as a model strain. The cultivation achieved up to 9 gL^{-1} and ≈ 5.1 gL^{-1} biomass concentrations at optimised conditions for both phototrophic and heterotrophic cultivations respectively. A single-use transparent bag was also investigated and the conditions for scale-up identified. Also, the application of ultra scale-down devices for product recovery was studied for unit operations such as flocculation, centrifugation, and filtration. However, high supernatant clarity of >99% was achieved at low centrifugal force ($V_{lab}/ct\Sigma = 1.7e^{-7}$) compared to 96% obtained in the unflocculated sample. In addition, ongoing scale-up studies will help to develop predictive tools to improve process performances at large-scale. The bioprocess development at milliliter scale minimizes costs, risks of scale-up and reduces product's time to market.

Considerations for downstream processing of microalgae and thoughts on commercialisation

Michael Graz and Almero Barnard, Neem Biotech Ltd

Commercialising algal products require a number of important considerations in order for the end product to fit purpose whilst being commercially viable. This presentation will touch on some of the considerations algae producers need to make when designing their downstream process. We will also discuss Neem Biotech's capabilities and how we may help a producer to get a product to market.

ALGAECOM: mining and optimizing microalgae biomass for novel cosmeceuticals

Emmanouil Flemetakis[1], Nikolaos Labrou[1], William Helbert[2], Konstantinos Gardikis[3], Lalia Mantecon[4] and Carlos Unamuzanga[4]

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Microalgae have the potential to revolutionize biotechnology in a number of areas including nutrition, aquaculture, pharmaceuticals, cosmeceuticals and biofuels. AlgaeCom project seeks to exploit the microalgae biodiversity, as a source for state-of-the-art high-added-value cosmeceutical ingredients. Towards this goal, we bring together two Academic (Agricultural University of Athens, AUA and Centre National de Recherches Scientifiques CERMAV-CNRS) and two Industrial partners (APIVITA SA and FITOPLANCTON MARINO) from Greece, France and Spain. In order to achieve its goals, the project is combining both basic and applied research in the fields of –omics technologies, biochemistry and applied biotechnology in order to: i) Develop catalomics, metabolomics and glycomics resources for marine and freshwater microalgae as a valuable source of novel high-added-value cosmeceuticals such as polysaccharides, enzymes and small molecule metabolites, ii) In vitro functional characterize microalgae cosmeceutical ingredients using human epidermal cell lines and in vitro skin model, iii) Develop and optimize application-based microalgae culture systems at different scales and optimize culture conditions for higher production rate of desired products, iv) Develop analytical molecular diagnostic tools for real-time monitoring of the microalgae carbohydrate and polysaccharide metabolism in large-scale cultures, v) Develop, formulate and in vitro evaluate a new range of cosmetic products based on microalgae extracts or isolated compounds. In addition, during its implementation AlgaeCom successfully integrated advanced research with an efficient mechanism for transfer of knowledge, training and dissemination which contributed to the reinforcement of the collaboration between academia and industry in Europe.

Biosurfactants and Bio-emulsifiers from Algae

Pattanathu Rahman, TeeGene Biotech Ltd.

The terms biosurfactant and bioemulsifier have often been used interchangeably to describe surface active biomolecules. However, it is important to note that there are marked differences between them especially based on their physico-chemical properties and physiological roles.

Although bioemulsifiers and biosurfactants are both amphiphilic in nature and are produced by a wide range of microorganisms, each exhibit characteristic roles in nature. These microbial surfactants have recently received increased scientific attention due to their unique characteristics relative to chemically derived surfactants. Their unique features include; non-toxic, biodegradable, biocompatibility, efficiency at low concentrations and their synthesis from natural substrates under mild environmental conditions. The combination of polysaccharide, fatty acid and protein components in bioemulsifiers confers upon them better emulsifying potential and ability to stabilize emulsions. It is also important to note that some efficient bioemulsifiers consists of only polysaccharides and proteins. On a general note bioemulsifiers have been associated with a number of potential applications including: remediation of oil polluted water and soil; enhanced oil recovery and clean-up of oil contaminated vessels and machineries; heavy metal removal, formation of stable emulsions in food and cosmetics industries and therapeutic activities (antibacterial, antifungal, pesticidal and herbicidal agents). Solar energy in the production of polysaccharides has been generally overlooked, despite high product yields and wide variety of polysaccharide production. However, due to current market demand for alternatives to synthetic surfactants and emulsifiers, the production of polysaccharides with surface active properties is attracting the attention of researchers. Oceanic biological surface active compounds still represent a major untapped and unexplored area of research. Algal EPS represent a huge range of structures. They are high-molecular-weight structures (10-30kDa) which encompass homopolymeric and heteropolymeric compositions. EPS structure varies widely between different genera of algae and is generally considered to be related to the environmental conditions on the organism. They are gaining much attention in relation to potential bioemulsifier properties. In particular, the green micro-algae *Dunaliella salina* and red algae *Porphyridium cruentum* are receiving attention as robust EPS producers with industrial application. They are highly biodegradable and have low toxicity. There is also an abundance of raw materials for the production of these molecules and they are highly biocompatible. Biosurfactants and bioemulsifiers share many environmental advantages, over their chemically synthesised counterparts.

Invited speaker biographies

Tracey Beacham (PML)

I am a molecular biologist and biochemist with experience working in both academia and industry. My PhD was on the modification of lipid pathways in transgenic wheat, to isolate the effects of climate change on this important crop. This was followed working for several years in industry (British Biocell International) developing novel in-vitro diagnostic tests. I returned to academia as a Postdoctoral scientist at Cardiff University studying chromosome dynamics in Saccharomyces cerevisiae. This combination of experience has proven valuable in my current roll here at PML; I have been involved in a collaborative 5 year BBSRC project aimed at an integrated approach to the cost-effective production of biodiesel from photosynthetic microbes. This has focused on genetic manipulation of marine microalgae to increase lipid productivity and improve the fatty acid profile for biofuel production via the development of micro algal genetic tools and isolation techniques.

Carole Llewellyn (Swansea University)

Carole Llewellyn is an Associate Professor in Applied Aquatic Bioscience at the University of Swansea where she leads research on algae covering their role in the environment and how they can be useful to society. This includes research on production of metabolites under different environmental conditions and on algal:bacterial interactions. Her background is in algal chlorophylls, carotenoids and UV sunscreens with recent interests in the application of metabolomics.
Before joining Swansea University, Carole was employed by the Natural Environment Research Council (NERC) at Plymouth Marine Laboratory for over 20 years where she led fundamental and industry led research focussed on the marine environment and blue biotechnology.
Carole has published >50 papers, book chapters and is an editor of a book on pigments. She is a recently selected member for the Royal Society of Chemistry Environment, Sustainability and Energy Division Council.

Tom Bibby (University of Southampton)

Tom Bibby is an Associate Professor at the University of Southampton. He works at the National Oceanography Centre and leads projects studying the role of phytoplankton (microalgae) in the ocean system and developing methods and techniques for their use in biotechnology. He leads projects funded by NERC, the Carbon Trust, a BBSRC/NSF initiative, and recently was awarded a BBSRC PHYCONET award partnering with Algenuity to develop genetic tools in Dunaliella.

Vítor Verdelho Viera (A4F)

President of EABA, European Algae Biomass Association (www.eaba-association.org) Board member and Chief Development Officer in A4F-AlgaFuel, S.A. (www.a4f.pt). President of Necton, S.A. (www.necton.pt). Graduated in Physics and has more than 20 years of experience in Microalgae Biotechnology. His activities involved the management of research projects, technology transfer and new business development.

Vítor Verdelho has a background in Solid State Physics and Management, having worked in biotechnology projects for the last 20 years.

With A4F Vitor Verdelho is presently coordinating the European Project **BIOFAT** - BIOfuel From Algae Technologies (www.biofatproject.eu); He is managing the participation of A4F in other large European Projects. **DEMA** – Direct Ethanol from Microalgae (www.dema-etoh.eu); **PUFA-CHAIN** - The Value Chain from Microalgae to PUFA (www.pufachain.eu); **D-FACTORY** - The micro algae biorefinery (www.d-factoryalgae.eu); **PHOTOCOM** - Design and engineering of photosynthetic aquatic communities for sustainable industrial use (http://photocomm.ku.dk); **ALFF** - Algal Microbiome: Friends or Foes (www.sams.ac.uk/Algal-doctorate-EU-Marie-Curie/algal-microbiome-friends-or-foes-alff);
PHOTOFUEL - Biocatalytic solar fuels for sustainable mobility in Europe (no website available yet); Has finished the participation in: **GIAVAP** Genetic Improvement of Algae for Value Added Products (www.giavap.eu); **PROECOWINE** Development of a process to generate a novel plant protection product enriched with micronutrients to replace copper in organic viticulture - Fraunhofer Institute; **AQUAFUELS** Algae and aquatic biomass for a sustainable production of 2nd generation biofuels (www.aquafuels.eu). This set of projects envolves the 30 most relevant Companies, Universities and Research organizations in Europe.

A4F – AlgaFuel, S.A. is a bioengineering company able to devoted to design, build, operate and transfer large scale microalgae biomass production plants worldwide. The knowledge and experience in all production technologies provides the clients a unique development capacity. The company is now starting the operation of the largest and most advanced photobioreactor in the world with 1100 m3 capacity, able to operate outdoors all the year.

Michael Graz (Neem Biotech)

Having recently completed my 100th marathon, my time is divided between managing a pre-clinical research biotechnology company and rogaining & long distance running with my wife.
I have 18 years of experience in managing turnaround situations in corporate environments and establishing startups and developing services in corporate and SME organisations within the life sciences, medical and

industrial biotechnology and food sectors. I have assumed P&L responsibility as well as provided technical consultancy and advisory services to a range of clients, including governmental and intergovernmental bodies. This has taken me to live on 4 continents and to work across 5.

I received my first PhD in Anatomy and Human Biology in 1995 and my second in Pharmaceutical Chemistry in 2002. Between these I lectured and supervised under-and postgraduate students. In subsequent years, I have taken on the role of external examiner and have been a research associate in 2 research units. I am a Fellow of the Society of Biology and am the current Chairman of the Society's Western Branch.

Almero Barnard (Neem Biotech)

Biochemist by trade, I have spent my professional career in Research and Development, and management of commercial (industrial-scale) protein extraction and purification departments. Currently, I am part of the Research and Development team at Neem Biotech.

I have expertise in lab- and industrial-scale chromatography, filtration (Macro-, Micro-, ultra- and nano-filtration) and various other fractionation techniques. I also have expertise in assay development (Microtitre-based colorimetric assay), protein crystallization, electrophoresis and other analytical techniques. I obtained my undergraduate degree in Animal Biotechnology. After completing my Hons. in Biochemistry I worked in the Biotech industry and obtained my MSc. in Biochemistry in 2012. This was based on my work on the optimisation of a commercial (industrial-scale) enzyme extraction and purification process.

Pattanathu Rahman (TeeGene Biotech Ltd)

Dr Pattanathu Rahman, Founding Director of TeeGene Biotech, is a Senior Lecturer in Process Engineering and Biotechnology at Teesside University with 20 years of research experience on novel biotechnological approach in bioproduct development, and a recipient of a Society for Applied Biotechnology (SAB) Award of Excellence in Microbial Biotechnology. Dr Rahman has completed a doctorate degree in Microbiology at Bharathiar University. He has worked as Postdoc at the University of Ulster and Brookhaven National Laboratory (New York, USA) and attended 'Biopharmaceutical Spring School' at Fitzwilliam College, Cambridge University. He is leading the MSc Biotechnology course at Teesside University and founded TeeGene Biotech in Dec 2014. TeeGene is a University's spin out venture, has developed unique strains of bacteria which produce biosurfactants. TeeGene's unique way of processing the biosurfactants and bioemulsifiers means the company is able to scale production to meet the demands of industry ranging from detergents, cosmetics to biopharmaceuticals. The company is also developing links with the National Biologics Manufacturing Centre and National Horizon Centre, which are under development adjacent to Teesside University's campus in Darlington.

Poster abstracts

1. Identication, isolation and purification of two toxic Cyanobacteria of Al-Mactaa lake (Mostaganem, west Algeria)

Aicha Zellal* and Sidi Mohammed El-Amine Abi-Yad (Department of Biotechnology, University of Oran, Algeria)
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The frequent dominance of eutrophic freshwaters by cyanobacteria (blue-green algae) is of increasing concern because these organisms can produce several types of potent toxins (cyanotoxins). This paper presents the first data on the identification, isolation and purification of some cyanobacteria, collected from a eutrophic lac (Al-Mactaa, Mostaganem, west Algeria) from July to August 2014 and isolated strain cultivated under laboratory conditions. Images of the cyanobacteria specimens were analyzed and matched with cyanobacteria in authoritative references and literatures on cyanobacteria taxonomy. The result of the taxonomical analysis showed many different species of cyanobacteria like: Anabaena sp, phormidium-chalybeum, Spirulina sp, Oscillatoria sp, Calothrix sp, Microcystis sp, Chroococcus turgidus, Merismopedia sphagnicola, Aphanocapsa sp. Culture samples of cyanobacteria on the solid medium BG11and the successive subcultures helped purify two toxic strains of cyanobacteria (Aphanocapsa sp and Anabaena sp), cells were harvested by centrifugation, frozen, freeze-dried and kept at -20°C until further use.

2. Synthesis of antibacterial protein against Gram-negative bacteria in the green alga *Chlamydomonas reinhardtii*

J. C. Ramos* and S. Purton (Institute of Structural and Molecular Biology, University College London)
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The widespread antibiotic resistance among pathogenic bacteria increases the need for the development of new antibacterial drugs. Endolysins are antibacterial proteins produced by bacteriophages to digest the peptidoglycan layer of the bacterial cell wall for phage progeny release at the end of the lytic cycle. These enzymes target molecules that are essential for bacterial viability, so the development of resistance is very rare. Therefore, endolysins have potential as novel antibiotics. Eukaryotic microalgae are becoming rapidly recognised as an alternative platform for production of recombinant proteins over usual systems such as bacteria, yeast and mammalian cells, due to established techniques for foreign gene expression, inexpensive cultivation and ease of large-scale production. The aim of this work is to produce bacteriophage endolysins in the chloroplast of the green microalga *Chlamydomonas reinhardtii*. An endolysin that has been shown to be effective against both Gram-negative and Gram-positive bacteria was chosen from the literature and assigned the code “JR1”. The JR1 gene was codon optimised for the *C. reinhardtii* chloroplast and modified to encode a C-terminal HA epitope tag. The synthetic gene was cloned into an expression vector which has the *psa*A promoter/5’UTR driving gene expression, and the rbcL terminator. This vector was designed to allow the cloning of a toxic gene into *Escherichia coli*. The resulting plasmid was transformed into *C. reinhardtii* using a simple glass bead vortexing method. Insertion of JR1 into the correct location in the chloroplast genome and homoplasmicity were confirmed by PCR, and accumulation of the 22.7 kDa JR1 protein confirmed by western blot. Further work is being done to analyse the antimicrobial activity of JR1 against several species of Gram-negative bacteria including *E. coli*.

3. The chloroplast of *Chlamydomonas reinhardtii,* a potential niche for biological nitrogen fixation

Marco Larrea-Alvarez* and Saul Purton (Institute of Structural and Molecular Biology, University College London)
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The extensive use of industrially produced nitrogen fertilizers has been associated with environmental damage and human health deterioration. Hence, it is of crucial necessity to develop alternative sources of fixed nitrogen. Biological nitrogen fixation rises as a potential “eco-friendly” option. Microorganisms carrying out this process are able to catalyse the reduction of atmospheric nitrogen into ammonia. This process relies on a group of metalloenzymes bearing molybdenum, vanadium or iron in their catalytic cofactors. It has been suggested that transferring nitrogenase genes into crops might increase the overall productivity; nonetheless, this strategy must overcome recognised obstacles such as the enzyme liability to oxygen or its complex biosynthesis. Plastids and mitochondria, due to their high level of ATP and reducing power, have been suggested as possible locations for the heterologous enzyme. However, the apparent complexity of coupling two mutually exclusive processes (oxygen-producing photosynthesis and nitrogen fixation) in plants render imperative the search for alternative eukaryotic platforms for nitrogenase expression. The green alga *Chlamydomonas reinhardtii* has been widely used for genetic manipulation and protein production on the grounds of growth rate, containment and minimal requirements for cultivation, and the standardised techniques developed for transforming the chloroplast of *C. reinhardtii* are now routine procedures. Similarly, engineering complex biosynthetic pathways have been successfully achieved, which demonstrates the resilience of the chloroplast metabolic network. Therefore, we argue that this microalga might be capable of sustaining nitrogen fixation within its plastids.

4. Engineering *Phaeodactylum tricornutum* for the enhanced production of omega-3 long chain polyunsaturated fatty acids

M L Hamilton, R Haslam, J Warwick, M Allen, J Napier, O Sayanova (Rothamsted Research)

Omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play essential roles in human nutrition during neonatal development and in adults. Currently the main dietary source of EPA and DHA is marine fish. Depletion of wild fish stocks, pollution of the marine environment and increased aquaculture mean that sustainable sources of EPA and DHA need to be developed. Whilst dietary needs for omega-3 LC-PUFAs are met through consumption of oily fish, the primary producers of EPA and DHA are marine algae and for this reason, provide an alternative source of dietary fish oils. The production of high value products such as EPA and DHA from algae is expensive and interest in the metabolic engineering of algae strains to improve omega-3 LC-PUFA content is considerable.

To date no single algae strain has been identified that produces high levels of both EPA and DHA. *Phaeodactylum tricornutum* is a diatom that accumulates up to 30% EPA but very little DHA. We describe the metabolic engineering of the diatom *P. tricornutum* with an eight fold increase in DHA production. We also demonstrate that DHA production accumulates in triacylglycerol. Large-scale culture of the transgenic strain has been investigated and DHA production measured under a range of growth conditions. The results from this study demonstrate potential for this engineered strain in the commercial production of EPA and DHA.

5. An oral vaccine candidate for infectious bronchitis virus through chloroplast engineering of *Chlamydomonas reinhardtii*

Priscilla D. Rajakumar*[1], Paul Wigley[2] and Saul Purton[1]
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Infectious bronchitis virus (IBV) is prominent in countries with an intensive poultry industry. However, vaccination is only partially successful owing to the high cost and emergence of antigenic variants. Therefore, there is an urgent need for more effective and cheaper vaccines. The genetically tractable microalga *Chlamydomonas reinhardtii* could be an ideal candidate for the production of oral IBV peptide vaccines, because: i) it has high growth rates, is capable of rapid scale up, cost effective culturing and protein production; ii) it is generally regarded as safe to eat; iii) genetic engineering of the chloroplast genome (=plastome) allows high-level expression of recombinant proteins. The goal of this research is to investigate the production of IBV peptide vaccines in the algal chloroplast, and the efficacy of the engineered algae as a low-cost oral vaccine for poultry. A synthetic biology approach was taken to design a multi-epitope IBV vaccine gene based on optimised codons for the algal plastome. The gene was cloned into an expression vector designed to create a fusion with the protein adjuvant, Cholera Toxin B. A plastome mutant of *C. reinhardtii* defective in photosynthesis was transformed with the pCTB-IBV plasmid using a simple vortexing method and transformant colonies selected based on restoration of phototrophy. Integration of the gene was confirmed by PCR and Western blot analysis confirmed the accumulation of the fusion protein. A preliminary immunogenicity test was carried out by a research group at the University of Liverpool. Lyophilised CTB-IBV transformed *C. reinhardtii* was fed to day 0 chicks, which were culled on day 28. The sera obtained from this test will be analysed by western blot and ELISA. Work is being carried out to obtain *C. reinhardtii* strains expressing higher levels of this recombinant peptide vaccine. We are also exploring the potential of this technology to produce oral algal vaccine for the fish farming industry.

6. The effect of viruses on the marine alga *Ostreococcus tauri*

Sarah Heath & Sinead Collins (University of Edinburgh)

Viruses are the most abundant biological entities on Earth and those that infect algae (Phycodnaviruses) play significant roles in cell mortality, cycling of organic matter and horizontal gene transfer. Currently there is interest in using viruses to improve lipid extraction efficiency from algae used for biofuel production since they are able to penetrate the rigid cell wall.

My research focuses on the evolution of the unicellular picoeukaryote *Ostreococcus tauri* when it is cultured in the presence of viruses. The genome of this alga has been fully sequenced and its simple structure and ease to culture makes it an ideal model organism to study physiological processes. I am using susceptible and resistant strains of *O. tauri* to examine whether susceptibility to viruses changes under different environmental conditions and whether there is a cost of resistance when cells are grown in stressful environments.

Increased knowledge of algae-virus interactions will allow us to better understand the role viruses can play in algal cultures, which could enable them to be used effectively in industrial cultivations of microalgae.

7. The microalga *Chlorella sorokiniana* as a platform for the production of tailored high-value oils

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Microalgae are promising biotechnological platforms: oil-rich biomass can be used for biofuels, and many species accumulate large amounts of high-value products such as pigments and long chain poly-unsaturated fatty acids (LC-PUFAs) including the well-known 'omega-3' oils which are proven to have nutritional benefits. However, there is often a trade-off in the production of these LC-PUFAs in that oil accumulation in the microalgae is often stimulated when under stressful growth conditions such as nitrogen deprivation. As such, growth rate is often dramatically reduced and the decrease in productivity can be a barrier to economic viability of industrial production.
My project aims to address this problem through a combination of genetic and metabolic engineering of the freshwater microalga *Chlorella sorokiniana* (UTEX 1230). This strain is of interest due to its intrinsic high growth rate, tolerance to high light intensity and growth in high temperatures up to 42°C. The work will first involve extensive characterisation of the natural lipid profile in a variety of growth conditions. Using a recently optimised nuclear transformation procedure, specific genes will then be inserted into the alga in order to upregulate LC-PUFA production.
This PhD project forms part of a BBSRC funded sLoLa grant 'Algal Oils by Design' in collaboration with groups at Rothamsted Research, University of Cambridge and University of Aberdeen.

8. Media optimisation studies and characterisation of nitrate uptake rate acclimation response in *Phaeodactylum tricornutum* from a bioremediation perspective

Víctor Sánchez Tarré[1], Matthew Davey[2], Louisa Norman[2], Brenda Parker[1]
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Drinking water purification plants utilise anion exchange columns to remove nitrate in order to meet criteria for safe supply. The waste by-product from column regeneration is brine water with an elevated nitrate concentration. Disposal of the brine without treatment is undesirable as it increases the burden on wastewater treatment and represents a loss of valuable nutrients. *Phaeodactylum tricornutum* is a diatom that can tolerate higher levels of nitrate than normally present in growth media. We wish to exploit this feature for bioremediation of the concentrated brine wash waste stream. Culturing *Phaeodactylum* in 24 well plates, we have utilised DoE techniques to optimise nutrient formulation for optimised biomass production. Additionally, we have studied *Phaeodactylum*'s acclimation response to nutrient surplus and deficit over the course of 8 weeks. By looking at the change in maximum growth rate (μmax), doubling time (t_d) and substrate coefficient (Ks) for NO_3^- in F/8, F/2 (control) and 2F media, we have characterised acclimation to different nutrient environments. To support these results, proteomic studies and qPCR experiments have been performed to understand patterns in gene expression over the prolonged exposure under different nutrient regimes. Combining experimental results with values extracted from the relevant literature, we have modelled a bioprocess using *Phaeodactylum* to recover valuable nutrients from brine waste and to produce value added algal biomass. This research lays the groundwork for further media optimisation alongside examination of nitrate removal efficiency studies under UK conditions.

9. Synthesis of recombinant antifungal enzymes in algal chloroplast

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Fungal diseases of plants have a major impact on crop production with an estimated loss of 125 million tonnes of food crops each year. This difference is in part due to the major fungal pathogens, *Phytophthora* and *Fusarium*. Current fungicide treatments involve the use of chemicals such as the azole group of compounds. However, a major concern is their harmful environmental effects and the emergence of high levels of resistance amongst the pathogens. In this project we are investigating a novel biofungicide strategy based on the production in green microalgae of lytic enzymes from potato that specifically target the pathogens by degrading their cell wall. These enzymes (chitinases and ß-1,3-glucanases) are natural components of the plant's pathogenesis response (PR). It has been shown that genetically-engineered plants that over-produce these PR proteins have significantly improved resistance. However, the creation and use of GM crops is expensive and complex, and raises major environmental and public acceptance issues. We are taking an alternative approach in which the enzymes are synthesized in the chloroplast of the harmless microalga, *Chlamydomonas*. Such microalgae can be grown at large-scale and cell extracts containing the enzymes could then be sprayed onto crops as a safe, biodegradable fungicide.

List of delegates

Abalde-Cela, Sara (University of Cambridge)	Llewellyn, Carole (Swansea University)
Ahmad Jamhor, Suhaiza (King Abdullah University of Science and Technology)	Lucas, Paul (University of Greenwich)
Al-Hoqani, Umaima (University College London)	Malin, Gill (University of East Anglia)
Allen, Mike (Plymouth Marine Laboratory)	Matthijs, Michiel (Algenuity)
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Barnard, Almero (Neem Biotech)	McKenzie, Douglas (Xanthella Ltd)
Bartasun, Paulina (Imperial College London)	Mokhtar, Noor Azlin (KAUST)
Beacham, Tracey (Plymouth Marine Laboratory)	Montoya, Haydee (Universidad Nacional Mayor de San Marcos)
Bibby, Tom (University of Southampton)	Ojo, Ebenezer (University College London)
Blanco Madrigal, Edgar (Anaero Technology)	Okorafor, Lawrence (Croda International PLC)
Border, David (DBCC)	Palm, R. (AF&F BV)
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Cortina, Angela (Avespa Holdings)	Pope, Matthew (Imperial College London)
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Feichtinger, Georg (Phycosera)	Sánchez Tarré, Víctor (University College London)
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Kelly, Ciarán (Imperial College London)	Theophilou, Stan (BlueBio)
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