Molecular tools for biotechnological exploitation of oleaginous yeast

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Generating new biofuels as replacements for fossil fuels is a major challenge for a sustainable future. The basidiomycete red yeast *Rhodosporidium toruloides* accumulates up to 70 % lipid per dry mass, which makes it of interest for biodiesel production (Liu and Zhao 2007). We have sequenced the complete genome of three strains of this yeast, annotated and analysed these with respect to lipid biosynthesis and breakdown, and performed transcriptomics analysis in lipid-inducing and non-inducing conditions. Development of this yeast as a renewable-fuel catalyst is dependent on developing molecular tools for genetic and metabolic engineering and the aim of this PhD project is to use our "omics" data to generate such tools and demonstrate their use for biotechnological exploitation of this yeast.

In this project the student will research and develop:
- Efficient and reliable DNA transformation procedures for *R. toruloides* (a protoplasting protocol is already developed in our laboratory).
- Integrative homologous recombination vectors, using codon-optimised selectable markers which we already have available.
- Targeted knockout/overexpression strains to enhance lipid production and secretion (e.g. as for *Saccharomyces cerevisiae*: Scharnewski et al. 2008).
- Proteomic analysis of engineered strains using GC-MS analysis of trypsinised protein extracts; we have demonstrated proof-of-principle proteomic analysis for this species.

This studentship will provide training in omics technologies, bioinformatics, molecular genetics and biotechnology.

Entry requirements: You should have a good degree (2:1 or above) in a relevant bioscience subject. You should have an enthusiasm for research in biotechnology/microbiology and the ability to work well as part of a team. You will join an established Microbial Biofuels Research Group at Exeter which has strong industrial links and access to excellent facilities.

Scharnewski M, Pongdontri P, Mora G, Hoppert M & Fulda M 2008 Mutants of *Saccharomyces cerevisiae* deficient in acyl-CoA synthetases secrete fatty acids due to interrupted fatty acid recycling *FEBS J.* 275: 2765-2778

Constructing synthetic protocells for the fabrication of functional nanomaterials

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**Background:** The use of nanomaterials has rapidly increased and their applications have emerged in a variety of commercial products including wound dressings, sunscreens and deodorants. Selenium based nanoparticles have properties useful in medicine, semiconductors and solar energy. These applications are of great economic and social importance for health improvement and “green” energy production. Developing synthetic methods for nanomaterial fabrication that utilise natural products, will have a beneficial impact on the environment, energy usage and human health. We have recently discovered a novel 95 kDa protein (SefA) secreted from the bacterium *Thauera selenatis* that is involved in the assembly of selenium nanospheres [1] during selenate respiration [2]. The SefA protein functions to assemble a selenium nanosphere (~150 nm in diameter) within the cell prior to secretion [1]. SefA has been
characterised, cloned and expressed in *E. coli*. Purified SefA has also been demonstrated to assemble selenium nanospheres *in vitro* [1].

**Aims:** We now aim to develop a synthetic system using SefA for the assembly of novel nanomaterials, specifically to yield innovations in fluorescent tag design (Se-based quantum dots) and drug delivery systems (nano-Se is non-toxic).

**Deliverables:** Artificial lipid vesicles will be synthesised and a mixture of SefA, intracellular reductant and a range of metal/metalloid (Se/Cd/Te) compounds added. The student will develop methods for synthetic nanoparticle fabrication, addressing key questions relating to how SefA controls nanoparticle size and composition. Experiments will be undertaken to determine which domains of SefA are essential for nanoparticle assembly. Truncated SefA peptides will be generated in *E. coli* using standard molecular techniques and tested in the synthetic cell. All fabricated nanomatetrials will be analysed using bioimaging and spectroscopic techniques. The student will also use a combination of biochemical and molecular methods to investigate the secretion apparatus in *T. selenatis*. It is envisaged that any translocation system identified will also be incorporated into synthetic vesicles to investigate mechanisms by which nanoparticles (of variable size) are translocated across biological membranes.

**Entry requirements:** Students interested in nanotechnology with degrees in Biochemistry or Chemistry are encouraged to apply.

**References:**

**Acid tolerance as a mechanism for environmental persistence and pathogenesis in *Campylobacter jejuni***

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*Campylobacter jejuni* (*Cj*) is the leading bacterial cause of diarrhoeal disease worldwide. Acid tolerance is critical for survival of *Cj* in both the environment and also as a mechanism of pathogenesis. In the environment *Cj* is exposed to low pH during survival within *Acanthamoeba castellanii*. *Cj* is a commensal organism in the gastrointestinal tract of chickens and must survive the low pH environment of the chicken crop to colonise and persist in this host. The main route of human infection is through ingestion of contaminated food or drink and so the bacterium must survive low pH in the stomach in order to successfully cause infection. *Cj* are thought to enter macrophages where they must survive low pH within the phagosome in order to establish infection. Clearly acid tolerance is critical for survival of *Cj* in the environment and host, yet the reason why acid tolerance varies significantly between strains, and the mechanisms behind this variability, has not been characterized. In this project the student will determine and compare the acid tolerance of *C. jejuni* strains isolated from humans, poultry, ovine, bovine and environmental sources. In addition, the student will sequence the genomes of selected *C. jejuni* strains. For this they will use our in-house Illumina HiSeq 2000 sequencing facility. Using bioinformatics the student will identify genes associated with acid tolerance. Elucidation of the functions of these genes (for example by constructing and testing mutants) will allow us to determine their roles in acid tolerance and subsequently niche adaptation and virulence. We have recently developed a novel assay for assessing *C. jejuni* virulence (Champion et al, JID, 2010) and we are developing
a zebrafish embryo infection model to study the interaction of _C. jejuni_ with the host. These models along with _in vitro_ tissue culture will be used in this project. In the longer term this project will provide new insight into the molecular basis of environmental survival and disease of this important human pathogen. This project provides the opportunity to receive world class, state-of-the-art, multidisciplinary training in the very latest cutting edge scientific and biotechnological skills that have relevance to microbiology as a broad ranging subject. Specifically, the student will be given hands-on experience in a range of cross-applicable techniques, ranging from molecular microbiology techniques, genomics and bioinformatic analyses through to infection assays and large strain collection study designs and rationales. The student will be based in a research group of 8 post-doctoral, 10 PhD students and 2 technicians who are working on the molecular basis of bacterial disease but will also work extensively with bioinformaticians at the University to analyse genome sequence data.

**Unravelling the cell adhesion defect in Meckel-Gruber Syndrome**

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**Project summary:**
Autosomal recessively inherited diseases are an important cause of morbidity and mortality, particularly in communities where consanguineous relationships (relationships between relatives) are common. Worldwide, at least 1 billion people live in communities where consanguinity rates are >20% and malformation disorders are frequent. One common malformation is failure to close the neural tube, which forms the brain and spinal cord during embryogenesis. The disease is caused by a combination of genetic and environmental factors, and can be due to a defect in cell migration or cell fate determination. Neural tube defects also form part of more complex disorders. One example is Meckel-Gruber syndrome (MKS), a lethal autosomal recessive malformation syndrome that is the most frequent syndromic cause of neural tube defects. The disease has a 100% mortality rate and patients seldom survive longer than a few days.

The first MKS genes were identified in 2006; however, the function of the MKS proteins and how the mutations lead to the disease still remain unclear. The only known role for MKS proteins is in the formation of cilia, organelles of motility and sensing that project from the surface of most human cells. More recently, we and others have demonstrated that there is also a cell migration defect in MKS-mutant cells and that this may be explained by problems in cell adhesion to the extracellular matrix.

The aim of this project is to use fibroblast cell lines derived from MKS patients to investigate the cell biology of the cell adhesion defect. The project aims to answer the following questions:

1. **How do mutations in MKS genes affect adhesion to different components of the extracellular matrix?**
2. **Are formation and turnover of the focal adhesions that link the cell to the extracellular matrix perturbed?**
3. **How is adhesion signalling perturbed in MKS patient cells?**

In answering these questions, the student will gain skills in mammalian cell culture and cell biology methods, molecular methods, transcriptomics and expression analysis, live cell imaging, confocal and TIRF microscopy, and scanning and transmission electron microscopy.

**Background References:**

**Application criteria:**
Applicants should possess at least a 2.1 Honours degree, or equivalent, in an appropriate subject (e.g. cell biology, biochemistry, genetics, or biological sciences)

**The evolutionary ecology of fungal plant pathogens**

Dr Ivana Gudelj  
Professor Nick Talbot  

Fungal plant pathogens are frighteningly successful. They cause severe plant diseases and are responsible for huge agricultural losses.

Their unerring success rests on environmental adaptation. Predicting the interactions between plant pathogens and their environment is difficult, but it is the essential challenge in Evolutionary Systems Ecology on which this PhD project is based.

We will undertake this challenge by visualising fungal infections as complex ecosystems in which individual strains and species engage in a variety of structured ecological interactions. In particular we will manipulate, observe, model and dissect the interactions between fungi and their environment focusing on the following unresolved question: **Are more diverse microbial infections more severe?**

Taking an interdisciplinary research path, we will combine mathematical modelling and laboratory experiments using the economically important pathogen *Magnaporthe oryzae* that causes the rice blast disease. The approach will span the molecular, ecological and evolutionary scales, from genes to populations and ecosystems.

As evidenced by our previous work listed below, advances in the molecular and genetic understanding of fungi, the ability to study adaptation in the lab and theoretical developments in the modelling of microorganisms have made it both feasible and timely to unify these disciplines within a single research programme.

Ultimately, our goal is to generate fundamental new insights into the evolutionary success of economically important fungal plant diseases.

**Gudelj group relevant theory publications:**
- Understanding the limits to generalisability of experimental evolutionary models. *Nature* 2008; 455
- A mixture of “cheats” and “co-operators” can enable maximal group benefit. *Plos Biology* 2010; 8(9)
- Metabolic trade-offs and the maintenance of the fittest and the flattest. *Nature* 2011; 472

**Talbot group relevant experimental publications:**
- A P-type ATPase required for rice blast disease and induction of host resistance. *Nature* 2006; 440(7083)
- Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. *PNAS* 2009; 106(37)
● Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* 2010; 330(6010)
● Horizontal gene transfer facilitated the evolution of plant parasitic mechanisms in the oomycetes. *PNAS* 2011; 108(37)

**The molecular basis for the assembly of Type II/III capsular polysaccharides**

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This project will seek to understand how some bacteria assemble their capsular polysaccharides (CPS). These polysaccharides are extracellular macromolecules found on the exterior of many Gram-negative bacteria, and acts as a protective barrier against a wide range of external insults, and are often essential for the virulence of pathogenic bacteria.

The project will specifically examine the principal CPS (CPS-I) of the human pathogen and potential bioterror agent *Burkholderia pseudomallei*. This bacterium produces two proteins that are common to many capsule biosynthesis pathways, whose functions are not well understood. Both proteins are necessary for *B. pseudomallei* infectivity, but the exact manner in which they work is not yet clear. In this project, you will investigate the proteins at structural, biochemical and cellular levels, with the aim of obtaining a thorough description of the proteins, and their interactions with other molecules in the cell. The techniques that you will learn to do this will include gene cloning, protein expression and purification, protein-protein interaction analysis, structural studies (principally X-ray crystallography), bacterial mutation, and some cell biology. It should offer a rounded PhD, with opportunities to learn a wide range of techniques and interact with other groups. This position forms part of a larger team within Harmer group examining CPS-I, so there will be good opportunities for interaction with other scientists within the department.

**Dynamic inference of large biochemical networks – uncovering transcriptional circuits in the yeast *Saccharomyces cerevisiae***

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Mathematical modelling is becoming an increasingly important tool in the biosciences, providing a quantitative framework within which to interpret experimental results and uncover the design principles of biological circuits. This project will develop novel computational methods for inferring the dynamic relationships underlying transcriptional responses using a modelling method known as Hidden Variable Dynamic Modelling (HVDM). This will be applied to analyse the transcriptional circuitry that controls environmental responses in the yeast *Saccharomyces cerevisiae*.

Previous studies have demonstrated that HVDM can successfully identify transcription factor targets in large microarray data sets, matching the performance of traditional methods based on clustering gene expression profiles. Moreover, by explicitly assuming a simple generic model for transcriptional activation, HVDM also generates confidence intervals for kinetic parameters and the predicted gene expression profiles. In its current form, however, the method only functions optimally for groups of genes regulated by the same transcription factor, which is a serious limitation.

*S. cerevisiae* is probably the best studied eukaryote on the planet, and a wealth of information on environmental responses, at the phenotypic and expression level; transcriptional control and regulation of gene expression exists and is publically available (SGD). Additionally the tractability of *S. cerevisiae* ensures that predictions made by the HVDM modelling can be experimentally verified. In this project the student will:
1. Broaden the scope of HVDM modelling by incorporating multiple transcription factors.
2. Improve the computational efficiency of the HVDM algorithm by using nested sampling as the parameter inference method.
3. Modify the HVDM model(s) to cover a broader range of regulatory mechanisms, and to include other data sources e.g. transcriptional motifs.
4. Use HVDM to generate predictive models of the response of *S. cerevisiae* to combinatorially applied environmental stressors.
5. Test the predictions of the HVDM models experimentally.

The student will work jointly in the Haynes/Akman labs and will gain expertise in algorithm development, implementation and optimisation; mathematical modelling of biological systems and importantly of experimental biology. This cross-disciplinary training will ensure that the student is uniquely placed to take advantage of the huge opportunities that exist, and will continue to do so, in quantitative biology.

We are looking for ambitious individuals with mathematical knowledge to degree level. Experience in wet lab work would be a real bonus, as would an MSc in Computational/Mathematical/Numerical Biology. Informal enquiries should be made to Professor Ken Haynes (k.haynes@exeter.ac.uk) or Dr Ozgur Akman (o.e.akman@exeter.ac.uk).

**Assessing trophic transfer for dietary exposure to oestrogenic endocrine disrupting chemicals**

Dr Tetsu Kudoh  
Professor Charles R Tyler

Endocrine disrupting chemicals (EDCs) are of worldwide concern and have been shown to induce adverse effects in both wildlife and humans. A major group of EDCs are oestrogenic chemicals that are present in the aquatic environment and derived from human and animal wastes, from derivatives of fertilisers, plastic products and several other industrial products. We have shown that sexual development in wild fish living in UK rivers is disrupted by these chemicals. Almost nothing is know however about the trophic transfer of EDCs in the food chain and this is a major knowledge gap. The main aims of this PhD studentship are to establish the ability of oestrogenic EDCs to pass through trophic levels in the food chain and establish the health effect outcomes in fish. To do we the studentship will employ the use of a transgenic zebrafish we have created at Exeter that fluoresces green in tissue that have been activated by oestrogen. The GFP transgenic zebrafish line contains synthetic oestrogen responsive elements (EREs), and a Gal4ff-UAS mediated signal amplification system that is one of the most sensitive oestrogen biosensor fish available.

The student will determine the ability of oestrogenic EDCs to undergo trophic transfer into fish via the diet (live plankton and algae), and assess where these EDCs accumulate in the fish body tissues for this route of exposure to inform on potential health outcomes. The project will include some targeted molecular analyses and also the development of a transgenic rotifer (which can also be applied for monitoring oestrogens in the water). These detailed studies will provide an understanding on the relative risks for exposure of zebrafish to oestrogenic chemicals via the food chain compared with that via the water (our ongoing work).

The student will thus get training in wide range of experimental techniques and be involved in a very exciting area of current biological research with direct environmental application. (S)he will also benefit form working in a vibrant research team with all the required infrastructure, including a recently established £9M aquarium facilities. Ideally, the student will have a background in either ecology, embryology or molecular biology.
Sperm pHertility; are faster marine invertebrate sperm more susceptible to ocean acidification?

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Marine invertebrates adopt a vast array of reproductive strategies and demonstrate considerable variation in sperm morphology and physiology according to phylogeny, spawning habitat and fertilisation strategy. The combined influences of rising atmospheric CO$_2$ and environmental pollution are now fundamentally altering the physicochemistry of the seawater into which these animals spawn at a rate exceeding anything in the historical and recent geological record. Our research has demonstrated that ocean acidification (OA) and environmental contaminants disrupt sperm function by reducing sperm motility and respiration rates and inducing DNA damage for a number of broadcast spawning marine invertebrates. However strong species-specific differences in the level of response to these stressors have been observed, suggesting reproductive processes in some species will be more susceptible to environmental change than others.

The aim of this studentship is to investigate the role of sperm morphology and physiology in determining the level of environmentally induced sperm disruption as a result of ocean acidification and pollutant exposure. We aim to test the hypothesis that: ‘faster, shorter lived sperm are more susceptible to environmental disruption from ocean acidification and pollution than slower, longer lived sperm.’ You will conduct comparative studies of marine invertebrate sperm responses to CO$_2$ induced ocean acidification (pH ranging from 8.1-7.4) and an environmental pollutant in terms of their viability, motility and longevity using computer assisted sperm analysis and live/dead fluorescent staining. Electron microscopy and antibody staining techniques (for proton pumps and calcium channels) will be employed to characterise sperm morphology and biochemistry for each species. A range of marine invertebrates (polychaetes, echinoderms and molluscs) covering different ‘spawning environments’ and phyla will be studied. In situ (field) and mesocosm experiments will look at how sperm traits influence fertilisation rates for different spawning environments.

You will be trained and supported by an enthusiastic team of Bioscience researchers providing you with a highly employable portfolio of skills in an area of key global importance. Experiments will be run at Streatham (Exeter) using the new state-of-the-art aquarium facilities with input on experimental design and interpretation from colleagues at Tremough (Cornwall). There will be opportunities for wider travel during the project to collaborating partners across Europe. Applicants are required to have a good degree in a biological science and a strong interest in the marine environment. For further details contact Dr Ceri Lewis at c.n.lewis@exeter.ac.uk.

Understanding environmentally-driven chromatin dynamics in seeds and the control of seasonal behaviour

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Seeds are the largest form of human and animal nutrition on the planet, and high seed quality is a key goal of plant breeders and seed producers. In the wild plants and seeds use environmental information to synchronise their life cycle with the seasons, and our lab would like to understand how this process will be affected by environmental change. In addition, we would like to understand environmental affects on seed behaviour that present problems for seed production, so that we can help seed producers design rational strategies for improving their processes for the delivery of consistently high quality seeds.

Temperature often affects plants by impacting on the chromatin structure of key genes with roles in the control of developmental transitions, such as from seed dormancy to germination. In this studentship you will investigate the role of key chromatin modifications on transitions into and out
of dormancy, and understand their importance both for plants in wild situations, and for seed production. You will also interact with theoreticians to understand the role of these pathways in whole plant life history, and the behaviour of plants under future climate scenarios.

**Two-component signalling in *Pseudomonas aeruginosa***

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*Pseudomonas aeruginosa* is the third most commonly acquired hospital infection, and is particular problem for immunocompromised patients and those with the genetic disease, cystic fibrosis. Bacterial behaviour during infection is controlled by two-component signalling pathways (TCSPs) which detect environmental conditions and bring about appropriate responses\(^1\). *P. aeruginosa* has around sixty distinct two-component systems. A variety of published transposon mutant screens have identified several two-component signalling pathways that are either required for virulence and/or for survival in a range of different hosts\(^2\)\(^-\)\(^4\). While some of these are extensively characterised e.g. GacS/RetS/LadS\(^5\), relatively little is known about the function of the remaining systems. The aim of this study is to investigate the role of these uncharacterised two-component signal transduction systems. It is anticipated that one of the long term impacts of the enhanced understanding of virulence signal transduction that will emerge from this study will be to facilitate the design of novel strategies for controlling virulence.

**Entry requirements:** A strong first degree (at least an Upper Second Class Honours or equivalent) in a relevant biological/biochemical subject.


**The role of DNA methylation on sex determination and development in zebrafish germ cells**

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Sex differentiation in vertebrates is a complex process that results in the production of viable gametes within the gonads. In mammals, the presence or absence of SRY on the Y chromosome determines whether an individual becomes a male or female, respectively. However, for many lower vertebrates, including many fish species, sex chromosomes are absent and the genetic triggers for sex determination remain largely undetermined. Furthermore, the regulatory events coordinating the sexual development of the germ cells as they progress towards mature gametes are unclear. In mammals, epigenetic events play key roles in the sex determination and differentiation pathways, through a number of mechanisms including DNA methylation and histone modifications. These events remain poorly studied in particular for lower vertebrates, despite their fundamental importance. This studentship will investigate the role epigenetic modifications (cytosine methylation) on the processes of sex differentiation and development in zebrafish. The project will benefit from the exciting developments in sequencing technologies and the sequencing facilities at Exeter (Illumina HiSeq sequencer) allowing for the investigation of the sex-specific patterns of DNA methylation at a single base resolution.

The outcomes of this project will provide invaluable information on the process of sex determination and differentiation in vertebrates with wide applications, from reproductive physiology and pathology, to environmental ecotoxicology and human health.
We seek a highly motivated student with a strong interest in applying modern molecular biology and bioinformatics tools to address fundamental questions in reproductive biology of fish. This innovative project will offer the candidate high quality training in a wide range of techniques, ranging from next generation sequencing to fish husbandry and to cell sorting and imaging, and will benefit from the state-of-the-art facilities and the world class research environment in fish reproduction at Exeter.

Complement Activation Makers for the Diagnosis of Infection in Acute Appendicitis

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Mr Ian Daniels PCMD and Royal Devon and Exeter Hospital Trust.

**Background:** We have been performing a series of clinical trials in collaboration with Mr Ian Daniels at the Royal Devon and Exeter Hospital and PCMD to look at the consumption and activation of the Complement cascade as part of the innate immunity response to recovery following surgical trauma, and the early detection of complications (1). We are preparing a trial to extend the study to the differential diagnosis of acute appendicitis (AA). AA is the most common abdominal emergency, accounting for more than 40,000 hospital admissions per year in England (2). Despite its frequency AA still represents a significant diagnostic problem as the signs and symptoms may vary from patient to patient and there is no one specific diagnostic test currently available. The treatment is surgical removal of the appendix as an emergency. Failure to recognise and treat appendicitis early results in significant morbidity and potential mortality (1). The diagnostic challenge is to discriminate between non-specific right iliac fossa pain and acute appendicitis. At present 15 to 30 per cent of appendicectomies performed ultimately reveal a normal appendix on histological examination (3), termed the ‘negative appendicectomy’ (NA) rate. These patients are exposed to unnecessary surgery with its associated risks. An estimated $741.5 million in total hospital charges resulted from admissions in which a NA was performed (3) in 2010.

**Aims:** Develop as series of Complement activation biomarker assays to monitor the immune response to infection and provide a differential diagnosis of Gram positive and Gram negative infection and diagnosis of AA.

**Deliverables:** The Complement activation profile will be correlated against the microbiological analysis of the appendix following removal to establish the differential innate response to Gram positive and Gram negative bacterial infection. The hypothesis will be tested statistically to calibrate the differential sensitivity of the innate immune response.

**Training:** The proposed PhD studentship will participate in a clinical trial providing the scientific testing resource for the pilot trial and subsequent clinical utility trial design. The student will use existing ELISA assays to measure the Complement activation markers and make comparable measurements on the in-house array reader platform. A full comparative analysis and statistical analysis will be performed in collaboration with Prof Rod Taylor from PenSTAT in PCMD to test the hypothesis of differential innate activation.

**References:**
[1] D Kahn, B. Jansen van Vuuren, E.M O’Hare, A. Perry, J. McGrath, IR Daniels and AM. Shaw


Sub-telomeric activation of novel plant-growth-promoting compounds in the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with rhizosphere pathogens in soil

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*Trichoderma* species are ubiquitous soil fungi that have attracted prolonged academic and commercial interest as biocontrol agents, due to their credibility as ecologically-friendly alternatives to fungicides in the control of plant diseases. Food security has risen to the top of political agendas, and the need to develop sustainable alternatives to costly fossil fuel fertilisers means that *Trichoderma* species are becoming increasingly attractive as biofertilisers also. Very few *Trichoderma* strains exhibit both properties - biocontrol and biofertilisation. However, *Trichoderma hamatum* GD12, a novel strain of *Trichoderma* isolated by Dr Thornton, displays the dual beneficial attributes of plant-growth-promotion and biological control of root-infecting pathogens. In previous research, we showed that genetic modification of GD12 significantly increased the P-G-P activity of the fungus, with concomitant hyper-secretion of water-soluble bioactive chemicals that stimulate crop root growth and canopy development. Furthermore, we showed that antagonistic interactions of GD12 with root pathogens in the plant rhizosphere not only provided complete protection from disease, but also enhanced the P-G-P activities of the fungus. Here, we hypothesise that co-culture of GD12 with rhizosphere pathogens leads to chromatin re-modelling of ordinarily silent telomeric secondary metabolite gene clusters leading to the production of novel bioactive compounds with previously uncharacterised P-G-P activities. To test this hypothesis the student will:

1. Generate single and double knock-out mutants of GD12 that differ in loss of one or more of a number of genes, identified in the GD12 genome by in-house Illumina sequencing, whose products regulate telomeric gene expression of secondary metabolites.
2. Use plant bioassays (crop plants and the model organism *Arabidopsis thaliana*), and soil microcosms, to investigate the effects of gene disruption on P-G-P activities and ecological and biological fitness of the mutants as biocontrol agents.
3. Undertake metabolomic characterisation (LC/Q-Tof) of the GD12 and mutant secretomes to identify novel bioactive P-G-P inducing molecules.

The key long-term objective of this research is to translate these discoveries into P-G-P products of agronomic importance. Identifying microbial activators of plant growth provides novel opportunities to enhance crop productivity through natural and sustainable agrochemical approaches.


**Campylobacter jejuni Niche Adaptation**

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*Campylobacter jejuni* is a leading cause of diarrhoeal disease in the world. However, the burden of human infection due to *C. jejuni* from different reservoirs is poorly understood, severely hampering our ability to track and control disease. In a preliminary study we have genome sequenced seven clinical *C. jejuni* strains, revealing marked genomic differences in function unknown (FUN), flagellin glycosylation and capsule biosynthesis genes. We have also previously
correlated flagellin glycosylation with colonization of poultry (Champion et al, PNAS 2005) and this finding indicates that there are genomic differences in C. jejuni strains that can be attributed to adaptation to their niche. In this project the student will determine and compare the genome sequences of a wide range of C. jejuni strains isolated from poultry, ovine, bovine and environmental sources. For this they will use our in-house Illumina HiSeq 2000 sequencing facility. This will allow the identification of source-specific genetic markers. Elucidation of the functions of these markers (for example by constructing and testing mutants) will allow us to determine their roles in niche adaptation and virulence. We have recently developed a novel assay for assessing C. jejuni virulence (Champion et al, JID, 2010) and we are developing a zebrafish embryo infection model to study the interaction of C. jejuni with the host. These models along with BIOLOG phenotyping will be used in this project. In the longer term this project will provide new approaches to tracing the sources of human infection and new insight into the molecular basis of disease. The student will be based in a research group of 8 post-doctoral, 10 PhD students and 2 technicians who are working on the molecular basis of bacterial disease but will also work extensively with bioinformaticians at the University to analyse genome sequence data.


**Synthetic Engineering of Host Metabolism for Increased Pathogen Resistance**

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Professor Orkun S Soyer  o.s.soyer@exeter.ac.uk

Viral and bacterial pathogens have major detrimental effects on human health and on the bioeconomy. Thus, both the natural emergence of pathogens and their potential engineering by hostile forces poses a significant biothreat challenge. To face this challenge and develop treatments against pathogens, basic research on understanding the host-pathogen interaction is essential. Using a synthetic biology framework, we plan to decipher pathogen manipulation of host metabolism and use this understanding to engineer the host metabolic network towards higher resistance. In this project, the student will initially focus this approach on the metabolism of an Escherichia coli cell infected with the phage MS2. We have selected a bacterium-phage model for the studies outlined below, because the system is easily amenable to high throughput screening and because a simple model (flux balance analysis or FBA) of cellular metabolism describing the bacterium MS-2 phage interaction. Using an existing metabolic model of this system and applying a novel metabolic analysis approach that we have developed, the student will predict the key enzymes in the host metabolism that impair MS2 growth. They will then experimentally implement these predictions and engineer a synthetic E. coli that is more resistant to MS2.

To date, there have been several synthetic biology studies concentrated on redesigning microbial metabolism [1-3]. These successful projects blended engineering principles of modelling and optimisation with genetic manipulation. Using stoichiometric models of cellular metabolism (flux balance analysis or FBA) [4] it is possible to develop a holistic, predictive model of cellular metabolism. The student will build an updated FBA model using the latest available information, incorporating the latest E. coli metabolic model [5]. The student characterise the resulting combined model and test it against experimental data; growth of uninfected and infected E. coli. They will then use synthetic biology techniques to introduce knock-out and over-expression of specific enzymes involved in the key reactions predicted. We will assess the ability of these
mutants to support the growth of phage MS-2 using standard microbiological methods. We will look for mutants which are more of less permissive to bacterial growth.

This synthetic biology approach is extendable to other host-pathogen pairs including human cells and their intracellular parasites. It will allow the development of novel treatments against pathogens through the engineering of host metabolism and will provide fundamental insight into the metabolic component of host-pathogen interaction. In the final year of the PhD, we expect the student to start working on human cell infections and test the predictions from the bacterial systems.


Cocktail Effects of Emerging Endocrine Disrupting Chemicals on Reproductive Health

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Disruption of reproductive health by pollutants in wildlife and human populations has received major worldwide attention. Exposure to chemicals that alter hormone systems, so-called endocrine disrupting chemicals (EDCs) has been shown to induce reproductive abnormalities including reduced sperm counts and quality in males. We have identified a suite of oestrogenic EDCs that accumulate in fish exposed to waste water treatment works (WwTW) effluents and proven they play a significant role in the feminisation of wild fish in UK rivers. Recently however, it has been discovered that chemicals that bind to and block the androgen receptor (anti-androgens) are widespread in UK surface waters (they are principally germicides) and chemicals with anti-androgen activity can induce a range of reproductive malformations too. There are furthermore anti-inflammatory pharmaceuticals (nonsteroidal anti-inflammatory drugs NSAIDS), that are also very widespread in the aquatic environment (and readily accumulate in exposed fish) that can act as anti-androgens reducing prostaglandin (PG) biosynthesis, that are fundamental for testis development. It is highly likely therefore these different types of EDCs will have combined effects on male reproductive development. The potential for combination effects of chemicals has serious consequences for the health of fish in UK rivers as the risk assessment of effluents is based on exposure to oestrogens alone, and does not take into account the aggravating effects of co-exposure to anti-androgens.

The main scientific aims of this PhD studentship are to establish the combined effects of key emerging EDCs, including pharmaceutical drugs, on reproductive health in the zebrafish model, and to include the application of state-of-the-art genomics, which in turn will better inform the risk assessment process.

This exciting PhD studentship will provide a very rich training in a wide range of techniques in in-life exposures, molecular biology (including, targeted q RT-PCR and next generation sequencing), physiology, ecotoxicology, and bioinformatics, using a model system. The student will benefit from working within a large, vibrant and interdisciplinary team that is well resourced and includes a recently established state of the art £9M aquarium facility. The student will have opportunity for travel to (and research in) other laboratories nationally/ internationally through ongoing collaborations (these include with other academic units, research institutes, government and regulatory bodies and industry).

Modelling early eukaryotic evolution using biogeochemical data – a systems approach

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It is commonly assumed that the ancestor of all eukaryotes was aerobic and acquired a mitochondrion when oxygen first rose to significant levels in the atmosphere. However, although atmospheric oxygen concentration rose in a ‘Great Oxidation’ of the atmosphere around 2.4 billion years ago, it only reached 1-10% of the present level, and the deep oceans remained largely anoxic and either iron-enriched or sulphidic until 580 million years ago, when the eukaryotic radiation was well underway. This new picture could explain the widespread distribution of anaerobic biochemistry in every eukaryotic supergroup.

This project will provide training in handling large-scale datasets of various strategically-chosen anaerobic eukaryotic taxa. We have the genomes of several key organisms and proven collaborations to provide access to other key genomes which have been published, or are soon to be submitted to high impact journals. Training will also be provided to use the Proterozoic Earth system model to explore the chemical composition of the ancient ocean, with particular emphasis on availability of different trace metals. This will be matched to the anaerobic biochemical potential of representative genomes, to arrive at a novel synthesis of these inter-disciplinary fields.

Professor Lenton is an expert in the coupled evolution of life and the planet and has recently developed an Earth system model of the Proterozoic Eon in which eukaryotes evolved. This model includes a 3-dimensional ocean with sediments and many geochemical tracers that can be matched to data recorded in ancient rocks to reconstruct the environments in which eukaryotes evolved. Van der Giezen is an expert in anaerobic eukaryote biochemistry and evolution and has recently reviewed energy metabolism in anaerobic eukaryotes. This is a good time to combine our expertise on the interplay between anaerobic environments and eukaryotes through a crucial interval of Earth history. We envisage several good quality publications, given that ancient ocean redox chemistry and anaerobic eukaryotic biochemistry have both been the subject of a string of high impact papers over the last ten years or so and we have been involved in several of these.

Suggested reading:

**The role of centrosome-associated nucleic acids in cell function**

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Centrosomes are conserved organelles that allow animal cells to, amongst other things, form a bipolar mitotic spindle capable of organizing cell division; as such, their dysregulation is implicated in many human diseases including cancer. Like mitochondria and chloroplasts, they duplicate independently of the nucleus, but unlike these other organelles, do not possess their own genome. The prevailing view is therefore that centrosome duplication is brought about through a protein-based template, rather than through using nucleic acids. However, recent work in model organisms conclusively demonstrates that centrosome-associated RNA (cnRNA) exists, and that representative RNAs localize to the centrosomal region of cells.

This project aims to find out what these cnRNAs are, and what they do in the cell. Using the model organism *Drosophila melanogaster* (the fruit fly), you will purify centrosomes, extract RNA and identify them, using our state-of-the-art next-generation sequencing platform. You will then
use Bioinformatics to analyse the results, allowing you to predict the function of groups of these RNAs. You will then produce individual cnRNAs, fluorescently label them and inject them into fruit fly embryos, using confocal microscopy to investigate their dynamic localization within the cell. You will use this knowledge to isolate mutant flies lacking these RNAs, characterizing the effect that mutating them has on cellular function.

These minimal-risk, data-rich studies will answer a fundamental biological question and could potentially change the way we view the cell.

The Wakefield Lab: The Wakefield lab is a vibrant, stimulating environment, investigating the principles of cell division (www.thewakefieldlab.com). Current members have a wide range of skills, with individual expertise in biochemistry, proteomics, Drosophila genetics, cellular imaging and image analysis, molecular biology and bioinformatics.

Modelling viral impacts on marine ecosystems

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This project will utilise state-of-the-art computational models to study the impacts of viruses on marine ecosystems. Viruses are the most abundant replicating entities in the oceans and are implicated in a wide variety of physiological, genetic, ecological and biogeochemical processes in marine ecosystems. Quantifying the net impacts of viruses on the biological pump of carbon from surface to deep ocean and understanding how these impacts will be affected by climate change are key challenges for understanding the marine carbon cycle.

Addressing these challenges will require the development of computational models of marine ecosystems that have sufficient biological complexity to handle microbial and viral processes. New metagenomic datasets are illuminating the coevolutionary interactions between viruses and marine microbes (e.g., showing how phage predation creates metagenomic islands in marine prokaryotes and how the resulting diversity facilitates coexistence of phage and cyanobacteria). New modelling approaches are needed that can use these valuable new data sources to link microbial and viral ecology to ecosystem dynamics.

The Earth System Science Group at University of Exeter has pioneered an evolutionary, trait-based modelling framework (the EVE model) that links microbial ecology to marine biogeochemistry (http://lifesciences.exeter.ac.uk/research/essg/projects/eve/). The EVE model is scalable from a 0D chemostat to a full 3D ocean circulation model, allowing study of local, regional and global processes. Current work within the group is developing a coevolutionary model of host-virus interactions in a simplified chemostat environment. The general aim of this project is to utilise and extend these models to study the impacts of viruses on the marine environment. Within this theme, there is considerable scope for the student to choose their own research direction to suit their own interests.

The student would be based in the Earth System Science Group in the College of Life and Environmental Sciences at University of Exeter (http://lifesciences.exeter.ac.uk/research/essg/). The student would enter a collaborative working environment offering excellent training in advanced numerical modelling, gaining core knowledge and expertise in marine biogeochemistry, microbial ecology and Earth system science. The project will require a strong background in mathematics, computer science, physics, or another numerical discipline. Additional background in molecular biology, microbiology, ecology, evolution, or environmental science would be an advantage, but is not essential.

Further reading:

Oceanic production of calcium carbonate (CaCO₃) is a major component of the global carbon cycle, and marine carbonate sediments provide invaluable insights for interpreting changes in ocean chemistry, benthic and pelagic ecology, and climatic change in the past, as well as modelling predictions for the future. In the open ocean, carbonate production is attributed primarily to marine plankton (mainly by coccolithophores and foraminifera). However, marine fish also produce calcium carbonate crystals in their guts which are excreted at very high rates. This gut process has significant implications for various physiological functions in marine fish (including acid-base and ion regulation, respiratory gas exchange). Importantly, the excreted carbonates have also recently been recognised as a major but previously unrecognised component of the global inorganic carbon cycle (Wilson et al., 2009 - Science). In addition, for tropical marine fish we have recently demonstrated that fish represent an entirely new (to science), direct and quantitatively significant source of fine-grained carbonate sediment within shallow tropical marine settings (Perry et al., 2011 - PNAS).

This PhD project will make steps towards a more global understanding of the role of fish both in the oceanic carbon cycle and carbonate sediments. This will be done by integrating physiological studies of common fish species from temperate water environments, with morphological and compositional analysis of the carbonates both produced by these fish and their occurrence within shallow marine carbonate sediments across the same temperate range. The successful PhD student will produce the first comprehensive dataset on the carbonate products of temperate water fish species. This data will be used to develop novel models, based on ecological data on distribution of fish biomass from temperate regions, to examine differences in total fish carbonate production and the likely fate of these carbonate phases. Training will encompass a multidisciplinary set of laboratory and field skills towards a topic of global environmental importance. Lab studies will involve collection of CaCO₃ crystals from a range of common marine species within the UK, held at 10-20 °C. Fieldwork will include sampling CaCO₃-rich sediments from candidate sites around UK coastal waters. Chemical analysis of bulk crystals from fish, and ambient sea water, will be analysed using high precision and custom-built seawater pH and infrared DIC (dissolved inorganic carbon) systems, automatic titration and ion chromatography equipment. Crystal morphology and individual crystal mineralogy and chemistry will be studied using SEM and SEM-linked energy dispersive X-ray (EDX) microanalytical facilities.

References: