Fully funded (BBSRC, UK) PhD position (4 years)

at University of East Anglia and Rothamsted Research, United Kingdom

"Enhancing the production of omega-3 polyunsaturates in marine microbes"

The primary producers of "fish oils" are marine phytoplankton that form the base of the aquatic foodweb. One alternative source of our ever-diminishing stocks of fish and fish oil is via the cultivation of these microbes. Unfortunately, these algae, of which diatoms are the dominant class, only accumulate oil during specific stages of their life-cycle and/or under nutritional states which are incompatible with the required high density of growth and target fatty acid profile. In addition, the endogenous levels of desirable fatty acids such as eicospantaenoic acid (EPA; 20:5, n-3) and docosahexaenoic acid (DHA; 22:6, n-3) are usually relatively modest (in the range 10-35% of total fatty acids) and therefore present an opportunity for enhancement. The Napier group at Rothamsted has a proven track-record in the identification and manipulation of the omega-3 LC-PUFA pathway in transgenic plants and yeast, and this expertise will be used to synergise the recent establishment at UEA by Mock of a robust diatom transformation system. Thus, this project will break new ground in the area of metabolic engineering of lipid metabolism in diatoms. The project will comprise of a number of independent workpackages as follows. The primary focus will be the manipulation of the diatom Thalassiosira pseudonana, which is rich in EPA but contains only low levels of DHA. The Napier group has already published a full characterisation of the T. pseudonana genes required for LC-PUFA biosynthesis (Tonon et al., FEBS J., 2005). WP1. Enhancing the levels of DHA in T. pseudonana. Two additional enzyme activities required for the conversion of EPA to DHA will be introduced in the diatom by biolistic transformation under the control of a constitutive promoter. Transgenic cells will be recovered via selectable markers and evaluated for the presence of altered fatty acid profile. In the case of this and subsequent WPs, the lipid profile will be determined by a state-of-the-art lipidomic analysis present in the Napier group at RRES – this will be performed by the student, who will thus gain valuable experience in mass spectrometry and also the handling of large datasets. WP2. As a reciprocal experiment to WP1, antisense RNA will be used to knockdown the endogenous levels of specific transcripts for components of LC-PUFA biosynthesis in T. pseudonana. Specifically, antisense will be used to silence either the Δ6-desaturase (which will block the synthesis of EPA and DHA) or the Δ5-elongase (which will block the synthesis of only DHA. These proposed experiments would define (for the first time) the role of PUFA s in the lifecycle of the diatom. Again, the lipid composition will be subject to "global" definition. The significance of LC-PUFAs to membrane function and cell survival will be examined by studying the growth of the transgenic cells under different conditions (temperature, CO2, nutrient status) and will be further evaluated by microarray analysis using a custom T. psuedonana chip. WP3. Using a more global approach, we will seek to identify genetic components which control the synthesis and composition of lipids in T. pseudonana. Specifically, we aim to identify transcription factors (TFs) which modulate omega-3 LC-PUFA biosynthesis and the synthesis of neutral lipids. Using bioinformatic analysis of the well-characterised T. pseudonana genome sequence (http://genome.jgi-

psf.org/Thaps3/Thaps3.home.html) and also pre-exisiting in-house transcriptomic datasets to identify transcripts modulated by nitrogen limitation, candidate TFs which correlate with the synthesis of lipids will be identified. The endogenous transcript levels of these T. pseudonana TFs will be modulated by transgenic over-expression or antisense knockdown to determine their role in regulation of lipid metabolism, using the approaches outlined above.

This studentship will provide experience in a range of techniques, using a combination of both well-established techniques (molecular biology, transcriptomics) and also leading-edge technologies (diatom transformation, lipidomics). The focus of the IBTI Club will also give the student an excellent introduction into the reality of "Excellence with impact" and how biosciences can deliver benefits to the public.

Please contact Thomas Mock at t.mock@uea.ac.uk for any inquiries you might have about this PhD studentship. Start date is 1st of October 2010. Please feel also free to visit www.mocklab.com.