Report for MASTS funded work at SAMS – enzyme extraction (DMSO reductase).

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My PhD project is investigating the effects of climate change on biogenic sulphur compounds in tropical corals. There are different methods of analysis for one of these compounds (dimethyl sulphoxide, DMSO), with varying levels of precision and difficulty. I had decided to use the enzyme linked method developed by Hatton et al. (1994); this technique involves using a solution containing DMSO reductase enzyme to reduce DMSO to dimethyl sulphide, which can then be measured using gas chromatography. However, DMSO reductase is very costly to buy (5 aliquots = £750 for ~250 samples) and so in July 2014 an application was made to the MASTS biogeochemistry forum for the purposes of collaborating with Professor Angela Hatton at the Scottish Association of Marine Science, so that I could learn the enzyme extraction technique as well as extract sufficient enzyme for use during my PhD. This was kindly granted by MASTS and during the week of 27th April I worked at SAMS learning the protocol. We successfully extracted ~3ml of enzyme, which doesn't sound like a lot but ~50µl is sufficient for ~50 samples, so 3ml is an ample supply for my PhD!

The enzyme is harvested from the bacterium *Rhodobacter capsulatus* strain H123; ~25L is required and this had been done prior to my arrival as it takes about a week to achieve a sufficient quantity. On Monday morning (27th April), Susan and I met to discuss the protocol and what would be done each day, which was followed up by making the required solutions for each stage of the extraction. In the afternoon we harvested the bacterial cells using cross flow filtration and centrifugation – this process essentially serves to remove as many bacterial cells as possible from the media, leaving us with a concentrated amount (~2L). Tuesday was a long day (~11 hours!) with the ultimate goal of opening up bacterial cells (lysing) so that proteins could be extracted from the periplasm (this is where DMSO reductase is located) – there was a lot of centrifuging! We ran into a small problem when the centrifuge broke, but managed to source another one capable of spinning at 8000 rpm so the week wasn't doomed. Once we had extracted all proteins from the periplasm we needed to pass our sample through a phenyl sepharose column so that the DMSO reductase enzyme could be separated from other proteins – this was done on the Thursday with Derek Thomson from Glycomar. We then concentrated the sample using more centrifugation and by 4pm on Thursday afternoon we had ~3ml of pure DMSO reductase enzyme (we confirmed the enzyme by checking its absorbance on a spectrophotometer – as advised by Derek). I still need to go back for one day to run quality assays for activity and concentration.

Overall the week was immensely rewarding, enjoyable, challenging and inspiring! It was a completely novel technique for me and I learned a lot. I'd like to thank the MASTS Biogeochemistry Forum and SAMS for making this possible, and Susan Evans (SAMS) and Derek Thomson (Glycomar) for generously giving up their time and making my week so thoroughly rewarding.