MASTS VISITING FELLOWSHIP

Project Summary

3rd March 2016

Visiting Fellow

Dr Naoki Kabeya

Department of Marine Biosciences, Tokyo University of Marine Science and Technology (TUMSAT), 4-5-7 Konan, Minato, Tokyo 108-8477, Japan

Host institutions

Dr Oscar Monroig, Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

Dr David Ferrier, Scottish Oceans Institute, School of Biology, University of St Andrews, St Andrews KY16 8LB□, Scotland, UK

Dates of visit

August 2015 – February 2016 (6 months)

Introduction

This MASTS Visiting Fellowship (VF) sponsored the visit of Dr Naoki Kabeya, Tokyo University of Marine Science and Technology, Japan, to two Scottish research groups, namely Dr Oscar Monroig, Institute of Aquaculture, University of Stirling, and Dr David Ferrier, Scottish Oceans Institute, University of St Andrews. With common interests in the study of the polyunsaturated fatty acids (PUFA) in aquatic organisms, the MASTS VF was initially focused on the molecular mechanisms involved in PUFA biosynthesis in polychaetes, a group of invertebrates whose PUFA biosynthetic pathways had been barely explored.

Importantly, this MASTS-funded visit expanded beyond polychaetes and also covered species representing other metazoan lineages including molluscs and crustaceans. The results of this project have allowed us to gain insight into the contribution that polychaetes and other marine invertebrates have to primary production of PUFA in the ocean, in particular that of the so-called "omega-3" (or ω3) fatty acids, a group of PUFA with well established health-promoting properties in humans.

It has been widely accepted that primary production of PUFA occurs in the ocean by the action of single-cell microorganisms such as bacteria, microalgae and heterotrophic protists occupying low trophic levels in the marine food web. On the other hand, higher trophic level organisms such as fish are only able to utilise and to some extent convert the primary produced PUFA into long-chain (> C18) PUFA (LC-PUFA), some of the latter are regarded as essential compounds, ensuring normal growth and development. However, little is known about the pathways of PUFA biosynthesis in the levels between primary producers and fish, which are largely filled by invertebrates. Our primary hypothesis is that marine invertebrates possess active enzymatic machinery involved in the biosynthesis of PUFA and thus contribute to the overall production of ω3 in the marine environment. We initially tested this hypothesis in polychaetes using *Platynereis dumerilii*. We then confirmed that other invertebrate groups possessed putative orthologues of the gene isolated from polychaetes and then we extended our investigation to molluscs (*Patella vulgata*) and crustaceans (*Lepeophtheirus salmonis*). Thus, our specific aim for the MASTS VF project was the molecular and functional characterisation of genes encoding desaturases, enzymes with pivotal roles in PUFA biosynthesis as they catalyse the introduction of double bonds (unsaturations) into fatty acids.

Results

In silico retrieval of sequences of putative desaturases from genomic and transcriptomic databases available for *P. dumerilii*, *P. vulgata* and *L. salmonis* enabled the identification of a type of desaturase with high homology to desaturases typically found in microalgae, plants and fungi. Phylogenetic analyses carried out with the sequences from the above listed species as well as

others from previously studied desaturase genes and with well-known functions, suggests that the newly identified genes are indeed more closely related to microalgal, plant and fungal desaturases than they are in comparison to desaturases from vertebrates.

Using PCR-based methodologies, we isolated the coding regions of the target desaturase genes and prepared plasmid constructs to run functional assays in yeast. Thus, transgenic yeast encoding the coding regions of the *P. dumerilii*, *P. vulgata* (two genes) and *L. salmonis* desaturases have been grown in the presence of potential fatty acid substrates. Fatty acid analyses have not been completed yet, but we anticipate that the functions of the newly cloned desaturases enable these invertebrate species to biosynthesize PUFA *de novo* and that, in particular, some of them are very efficient in the production of ω3 PUFA. Upon confirmation, these results challenge the historical vision of marine microorganisms being the sole source of ω3 PUFA in the ocean and provides compelling molecular evidence supporting that marine metazoans are also primary producers of ω3 PUFA.

Networking with Scottish researchers

The MASTS VF to Dr Kabeya has allowed the establishment of a new collaboration between his home institution in Japan (TUMSAT) and both host institutions in Scotland, namely UoS and UoSA. In addition to the specific collaboration with the host researchers Dr Monroig (UoS) and Dr Ferrier (UoSA), Dr Kabeya has been also able to expand his network with other researchers in both host institutions and beyond. For instance, Dr Kabeya is currently conducting further phylogenetic analyses with Dr Filipe Castro (CIIMAR-Porto University, Portugal) in order to elucidate the evolutionary history of desaturases found within this project. It is expected that the collaboration initiated herein, with the host institutions and beyond, can be continued and expanded in the future.

Overall logistics during the MASTS Visiting Fellowship

The project was carried out primarily at the Institute of Aquaculture, School of

Natural Sciences, University of Stirling, under the supervision of Dr Monroig. Moreover, Dr Ferrier (host at UoSA) has facilitated key polychaete samples for this project, provided access to genomic and transcriptomic databases and aided Dr Kabeya during sequence retrieval. Other biological samples from *P. vulgata* and *L. salmonis* were provided by colleagues at UoS and CIIMAR-Porto University, respectively. Expenses related to the experiments were covered by ongoing projects related to the topic of the MASTS VF.

Significance and future prospects

The results obtained in this study strongly suggest that polychaetes, as well as other invertebrate lineages inhabiting the marine environment, possess desaturases enabling these organisms to biosynthesise PUFA *de novo*. A comprehensive description of the gene repertoire in key invertebrate species will facilitate our understanding on the molecular mechanisms underlying PUFA biosynthesis in marine invertebrates, and thus to elucidate their contribution to the overall production of omega-3 long–chain PUFA in the ocean. Furthermore, these studies can not only illuminate alternative and unusual biosynthetic pathways and metabolism, but will also provide insights to gene and pathway evolution, as well as being a resource that can supply potentially valuable molecular tools in the form of genes involved in PUFA biosynthesis and metabolism.

Acknowledgements

I (Dr Kabeya) am grateful to MASTS for giving me a great chance to work with Dr Monroig and Dr Ferrier in such a challenging and interesting project. I appreciate all of the kind support from staff members and students in the host institutions. It was a very productive visit and a good opportunity to establish a long-term and fruitful collaboration with these Scottish institutions. We have already started to draft a paper on the results obtained from this project. As requested, the MASTS funds will be acknowledged in any publication derived from the present proposal.