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Editorial Open Access

Marine Proteomics: Challenges and Opportunities

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Editorial

Marine invertebrates such as crustaceans, oysters, polychaetes and bryozones are widely distributed in intertidal and sediment ecosystems and have significant economic and ecological importance. These species serve as model organisms for settlement biology, biofouling, marine pollution, toxicology, climate change and ocean acidification research. Traditionally, marine biologists have studied them in the context of morphology and behavior. Ever since the technological advancement in proteomics and genome sequencing tools, researchers began to adopt such tools in marine larvae biology research [1]. However, the challenge is that none of these tools were applied to marine research before so there is a great deal of optimization of such methods has to be done. In addition, lack of genome information discouraged marine scientists to conduct proteomics studies. Cell culture methods have not been established and species specific antibodies are not yet developed. The direct use of larvae or adults increases the complexity of proteome. Shells and calcareous tubes of crustaceans and oysters and self-secreted mucilage or slime of polychaetes poses greater challenge for proteins extraction and purification protocols. Natural habitat conditions differ substantially thereby variation in data obtained is high.

Despite of challenges, in the past seven years, these limitations have been overcome by several research teams and have been successfully carried out proteomic investigations using marine invertebrates. Our own studies provided wealth of information on proteins/ genes and molecular pathways. Pei-Yuan Qian lab at the Hong Kong University of Science and Technology in Hong Kong has optimized and applied gel-based as well as gel-free proteomics tools to study larval settlement and metamorphosis and their response to antifouling agents. Our effort greatly improved protein extraction from larvae, juvenile and adults. The proteome complexity issue was addressed by optimizing sample pre-fractionation methods and iso-electric focusing (IEF) conditions. This enabled drastic increase in number of protein spots detected on gels. Two-dimensional gel electrophoresis (2-DE) based multiplex proteomics, sequential fluorescent staining, and approaches that allowed parallel analyses of total proteins, phosphoproteins and glycoproteins on single 2-DE gel.

Our research also demonstrated that phosphoprotein enrichment strategy is an effective method for the identification of phosphoproteins in larval proteome. Further, they managed to develop an in-house transcriptome databases by using next generation RNA sequencing for selected marine species and such databases freely available in public domain for other investigators. Thousands of transcripts were cataloged and used as a reference transcriptome for proteomic studies. This work has led to the application of shotgun proteomics tools in marine biology research. Label-free as well as labeled, isobaric tags for relative and absolute quantification (iTRAQ) quantitation and peptide OFFGEL fractionation methods have been established to obtain comprehensive coverage of proteome [1].

Before the availability transcriptome databases, raw data or mass spectra obtained from mass spectrometer was used to search against standard databases such as NCBI nr and SwissProt, to identify the homologous proteins. However, this approach failed to identify proteins that are not listed in the standard database. Most of the protein ID hits displayed as "hypothetical proteins" and were not specific to species of interest. Then researchers attempted to mine such missing proteins by applying search algorithms such as MS BLAST, de novo sequencing and close species homology search. In some studies combinatorial application of search algorithms successfully identified missing proteins. However, such methods have shortcomings such as short sequence tags and false positive identification. Recently, customized species specific transcriptome databases are available for many marine species and taking advantage of such database as reference genome for proteome analysis is the way forward. Marine researches are now started to use advanced qualitative and quantitative software such as PEAKS, Scaffold and Protein Pilot to measure the protein abundance more accurately.

Ever since the successful optimization of proteomic tools, several research teams identified thousands of proteins and transcripts in the larvae and juveniles of marine invertebrates. Some of them are found to be crucial for larval settlement and metamorphosis, reproduction, anti-fouling targets, and biomarkers for climate change and OA.

Donald Reish, Emirates Professor at California State University Long Beach collected polychaete worms, Neanthes arenaceodentata, in Los Angeles Harbor in 1964 and established the worm culture that undergone over 200 generations of reproduction without addition of new worms. He was never been able to look at the protein metabolism in worms. In a joint collaboration effort, we used proteomics methods to study these worms unique and rare reproduction pattern in nature where female die after laying eggs and male incubate the eggs and capable reproducing nine times. We identified several proteins which provided clue behind reproductive success of male worm [2].

Ocean waters are becoming warmer and acidic due to global warming and drastic increase in CO_2 in the atmosphere threating to entire marine ecosystem and public health. Therefore it is important to understand how marine organisms adapt and survive in such conditions. Thiyagarajan V and his team from Hong Kong University, Hong Kong, focused on what effect OA on larvae of barnacles and oysters. To document the effects of acidification, the team measured metamorphosis success in the larvae by exposing to hypoxic (low oxygen) conditions. They showed that hypoxia seemed to rescue the larvae from the negative effects of ocean acidification. In another study, they revealed proteomic plasticity enabled oysters larvae a short-term adaptation or acclimation to a near-future OA scenario. In a recently published work we exposed larvae to multiple stressors such as decreased pH, increased temperature and reduced salinity, and found that phenotypic plasticity of proteome appeared to be essential for survival in future acidic oceans [3]. Timothy Ravasi from King Abdullah University of Science and Technology, Saudi Arabia and his

collaborators sequenced the transcriptome and proteome of barnacle larvae, nauplii. Our work revealed the role of protein expression patterns in developing strategies to adopt and survive in the extreme Red Sea marine environment. Remarkable plasticity was observed in the proteome of two larval populations collected from Hong Kong coast and central Red Sea. Stress responses and osmoregulation proteins facilitated larval survival [4]. His lab is now focusing on transgenerational effect of high CO_2 on coral reef fish.

Collectively, optimization of proteomic tools and availability of transcriptome sequences greatly advanced marine larval biology research. Adopting such tools the marine proteome research has significantly expanded. Researchers have learned about how such tools can be applied to marine ecosystem studies. Large amount of transcriptome and proteome data is available for several marine species. Despite these successes, the challenges and limitations still exist. For example, full genome sequences not yet available for majority of the marine species. Protein array and knock down studies are not yet to be feasible for marine larvae. It is a call to the marine research community to look into such challenges. With this note, marine proteome research is still in the juvenile stage and yet be grown in many marine labs.

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