

Master Dissertation Project

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Lab/Institution: Mass Spectrometry - ITQB

TITLE: Impact of seawater acidification in starfish regeneration – a proteomic approach

BACKGROUND

The potential for regeneration has its maximum expression in echinoderms. Preliminary studies of regeneration in echinoderms were based on the determination of growth rates and on the morphological, histological and cellular basis of this phenomenon. More recently, some advances have been made in the characterization of the molecular mechanisms involved in the regeneration process of tissues and organs. Studies that have been developed in our laboratory focus on a star-fish species common in coastal areas of the North Atlantic and with high regeneration capability, the *Asterias rubens*. Behavioral assays allowed to observe differences in the regenerative capability of this species exposed to different seawater pH values. No information on the effects of ocean acidification, inherent to the global warming, are available for the pH homeostasis in echinoderms. Understanding the capabilities of acid-base regulation is important because of the dependence of the formation and maintenance of the calcium carbonate skeleton and, consequently the regeneration process of these species.

CF Franco *et al* *Proteomics* (2011) 11: 1359-1364; CF Franco *et al* *Proteomics* (2011) 11: 3587-92; CF Franco *et al* *Electrophoresis* (2012), 33, 3764–3778; C Franco *et al* *J Proteomics*, 2014, in press; C Franco *et al* *Proteomics* (2013), 13(3-4), 686-709 (Review article)

OBJECTIVES

The intention of the proposed theme is the identification of metabolic processes affected by exposure of *A. Rubens* to seawater with different pHs and the impact of environmental conditions on the regeneration of this species. A differential proteomic approach will be used for the detection of differentially expressed proteins in the coelomic fluid and its subsequent identification by mass spectrometry.

PROJECT DESCRIPTION

The process of sea-stars handling, including the induction of regeneration and tissue collection were made at the Sven Lowen Marine Station (University of Gothenburg, Sweden). Protocols have already been developed for the proteomic characterization of coelomic fluid.

Task 1: The protein extracts for each condition (intact and amputated specimens in different environmental conditions) will be obtained, quantified and digested with trypsin for LC-MS/MS analysis.

Task 2: MS and MS/MS data collection: The differentially expressed proteins between different groups will be quantified and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics.

Task 3: Protein identification and quantification data treatment: Profile data from the MS scans are transformed to m/z peak lists. Protein identification will be performed using the MS/MS spectra and submitting to a search engine using a general database. For quantification, all reimported peptides of an identified protein are included, and the total cumulative abundance is calculated by summing the abundances of all peptides allocated to the respective protein.

Task 4: Pathway analysis using the proteins identified with expression differences between each pair of sample groups

Task 5: Writing of Master Thesis

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										