Exploring the sexual phase of the diatom *Pseudo-nitzschia multistriata*: from genes to metabolites

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Abstract

Phytoplankton are an important component of the marine ecosystems since they are at the base of marine food webs and are responsible for about 50% of the oxygen production at a global scale. The knowledge of phytoplankton life cycles is important to understand the mechanisms regulating the dynamics of their populations in the natural environment but also to improve the capability to cultivate them and thus exploit their biotechnological potential. The model organisms chosen for my PhD project is a marine planktonic diatom, *Pseudo-nitzschia multistriata*, known to produce the neuro-toxin domoic acid (DA), the causative agent of Amnesic Shellfish Poisoning.

Pseudo-nitzschia multistriata is a pennate heterothallic species whose life cycle has been described and can be controlled in laboratory conditions. Cells of the opposite Mating Types (MTs) can switch from asexual to sexual reproduction after reaching a threshold size and it is very important that this process is finely synchronized and regulated to ensure its success. There is evidence of a chemical cross talk that mediates mating and some of the genes involved in the process were identified.

In this thesis, several aspects related to the reciprocal perception of the opposite MTs and the comparison a between a 'treatment' in which sexual reproduction was ongoing as compared to the 'control' of monocultures of single parental strains were untangled focusing on a set of genes involved in the process.

Several experiments illustrated in Chapter 2 were aimed at i) further elucidating the expression pathways of the target genes and ii) identifying a set of genes that could be used as marker genes for future isolation of putative pheromones. The results of the experiments suggest that in *P. multistriata* there are two constitutive pheromones called *MRP1* and 7488

produced by MT+ and MT-, respectively. Among these the gene 7488 was upregulated in MT- exposed to the culture medium in which MT+ was growing; this gene can thus be considered the as the molecular marker for a bio-assay to detect the fraction of MT+ conditioned medium containing the putative pheromone. The experiments carried out in this thesis also suggested that that the constitutive putative pheromone of MT+ perceived by MT- could be MRP1, a gene encoding for a small secreted protein, that is differentially expressed between opposite MTs and is highly induced during sex in the MT+.

In Chapter 3, I have illustrated the spatial pattern of a set of genes involved in the sexual phase using the large database of genes from the Tara Oceans expedition. The co-occurrence of MT related genes and one meiotic gene in nine TARA stations suggests that sexual reproduction was occurring in those sites. The spatial distribution of these genes was not uniform and this can be explained with differences in their basal expression levels and/or by the absence of species in which the genes are present.

Finally, in Chapter 4 I explored the difference between metabolites produced by the parental strains in vegetative growth in monoculture and by a co-culture of strains of opposite MT undergoing sex. The metabolomics analysis revealed that there were not exclusive metabolites distinguishing the vegetative phase and the sexual phase, but there were a number of mostly unidentified metabolites that increased their quantity in the sexual phase. The results of this thesis add novel information on several aspects concerning the mate perception of a planktonic diatom, elucidating hierarchical activation of genes during the sexual phase. Furthermore, with future identification of the constitutive cue of MT+, MT- gene expression changes in response to the first MT+ signal might be clearly elucidated using RNAseq. Last but not least, this work indicates that genes related to sex could be makers for sexual detection at sea, becoming a useful tool for ecological purposes.