

PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Ecologie, Géosciences, Agronomie, ALimentation »

GENERAL INFORMATION

Thesis title: Characterization of microalgae non cyanobacteria interactions for nitrogen fixation
Acronym: MABFIX
Disciplinary field 1: Ecology Disciplinary field 2: Agronomy
Three keywords: Microalgae, non cyanobacteria, nitrogen
Research unit : US2B
Name of the thesis director HDR (Habilitation thesis to supervise research) required: Tirichine Leila Email address of the thesis director: tirichine-l@univ-nantes.fr Name of the thesis co-director (if applicable): HDR (Habilitation thesis to supervise research) required: Email address of the thesis co-director (if applicable): Name of the thesis co-supervisor 1 (if applicable): Email address of the thesis co-supervisor 1 (if applicable): Name of the thesis co-supervisor 2 (if applicable): Email address of the thesis co-supervisor 2 (if applicable):
Thesis grant (funding origin and amount): ADEME+Région Pays de la Loire (not granted yet)
Contact(s) (mailing address and E-mail):
Recruitment process: Recruitment process depends on thesis funding. To select the corresponding recruitment process, please visit the EGAAL website here . This information is needed for proposal publication. <input type="checkbox"/> Doctoral school contest <input type="checkbox"/> Interview <input checked="" type="checkbox"/> Other (indicate) : ADEME half fellowship and Région Pays de la Loire half Fellowship

ED EGAAL

Direction : 65 rue de Saint-Brieuc – CS 84215 – 35042 Rennes Cedex – France

Tél : 02 23 48 52 75

Mail : ed-EGAAL@doctorat-bretagneloire.fr

Site Web : <https://ed-egaal.doctorat-bretagneloire.fr>

All sections must be filled. Once filled, please save the proposal form in pdf format using the following naming: Supervisor Name_Unit_Subject Acronym_EN.pdf

ED EGAAL

Direction : 65 rue de Saint-Brieuc – CS 84215 – 35042 Rennes Cedex – France

Tél : 02 23 48 52 75

Mail : ed-EGAAL@doctorat-bretagneloire.fr

Site Web : <https://ed-egaal.doctorat-bretagneloire.fr>

SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

Socio-economic and scientific context : (10 lines)

Nitrogen is an essential limiting element for the growth of living organisms. It is a major component of chlorophyll, the most important pigment required for photosynthesis, as well as amino acids, the main building blocks of proteins, and other important biomolecules, such as ATP and nucleic acids. The availability of nitrogen (N) limits biological production in large areas of the world ocean and is therefore closely linked to atmospheric carbon dioxide fixation and carbon export from the ocean surface. About 80% of the Earth's atmosphere is in the form of N₂ gas and only certain bacteria are able to fix it. It is generally considered that cyanobacteria are responsible for most of the nitrogen fixation in marine waters. However, this has recently been the subject of debate as cyanobacteria are not the only diazotrophs in the oceans. Recent molecular analyses indicate that non-cyanobacterial diazotrophs (NCDs) are also present and active. Indeed, an overwhelming dominance of various nifH amplicons, a nitrogenase-related gene, has been detected, linked to non-cyanobacteria, in particular to gamma and alpha proteobacteria. It was suggested that the distinct diazotrophic community of proteobacteria is attributable to oligotrophic conditions and that mutualistic interactions between bacterial nitrogen fixation and the release of dissolved organic carbon by phytoplankton may underlie oligotrophic ecosystems. Nitrogen fixation by NCDs may therefore have a profound impact on the global nitrogen balance, primary productivity and consequently CO₂ sequestration. No studies have been carried out so far on the interactions of proteo-type nitrogen-fixing bacteria with microalgae and our recent discovery of the presence of this type of interaction with a model diatom opens up promising new perspectives for understanding the molecular mechanisms of this interaction and the possibilities of applying these interactions to synthetic biology, where we can imagine the development of a more efficient and effective nitrogen-fixing system. This will permit the exploitation of microalgae without nitrogen fertilisers, which are a source of pollution and represent a significant cost, especially at a time when there is a shortage of fossil fuels and the dependence of Europe on Russian gas.

Assumptions and questions (8 lines)

We are interested in the host specificity for nitrogen fixation in the model diatom *Phaeodactylum tricornutum* in which we have discovered an interaction with proteobacteria that fix atmospheric nitrogen. We have several natural variants in this species that show a different response after inoculation with a nitrogen-fixing bacterium, suggesting the presence of specific compounds in the host that determine the specificity and efficiency of nitrogen fixation

Which genes and metabolites in the diatom are important for host specificity?

What molecules are secreted by host cells to induce and establish the interaction?

What are the epigenetic factors that regulate host specificity?

The main steps of the thesis and scientific procedure (10-12 lines)

The project will start with the confirmation of a screen already performed and its extension to other natural variants of *P. tricornutum*. This step consists of a global screen of all accessions of *P. tricornutum* in nitrate starved condition

Beforehand, the accessions must be made axenic by using a cocktail of antibiotics and verification using a peptone rich, as well as transmission microscopy after staining with DAPI, which allows the detection of bacteria if present.

The screen will identify compatible and incompatible interactions and a range of intermediate combinations.

The transcriptome, epigenome (DNA methylation as well as acetylation and trimethylation of lysine 27 of histone H3), metabolome and secretome of 3 combinations (compatible, incompatible and intermediate) will be analysed. The key genes determined and the functional characterisation of 3 key genes will be performed using a CRISPR cas9 genetic approach.

Methodological and technical approaches considered (4-6 lines)

Once the screening has been carried out by measuring the growth of the cells, the selected combinations will be confirmed by an Acetylene reduction assay which will allow the quantification of the efficiency of nitrogen fixation. Next, a global omics approach will be undertaken. From RNA seq, DNA methylation using bisulfite sequencing and investigation of the distribution of key histone marks using CUT and RUN, with the analysis of the metabolome and secretome.

Finally, the identified genes will be characterised by CRISPR cas9.

Scientific and technical skills required by the candidate

Skills in molecular and cellular biology. Knowledge in bioinformatics is an asset

THESIS SUPERVISION¹

Unit name: Unité en Sciences Biologiques et Biotechnologies (US2B)	Team name: Epigenomics of microalgae and interactions with the environment
Unit director name: Simier Philippe	Team director name: Tirichine Leila
Mailing address of the unit director: philippe.simier@univ-nantes.fr	Mailing address of the team director: tirichine-l@univ-nantes.fr
<p>Thesis director</p> <p>Surname, first name: Tirichine Leila</p> <p>Position: CNRS research director</p> <p>Obtained date of the HDR (Habilitation thesis to supervise research): 04/12/2014</p> <p>Employer: NRS</p> <p>Doctoral school affiliation: EGAAL</p> <p>Rate of thesis supervision in the present project (%): 100%</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 100% and 50%</p> <p>Number of current thesis supervisions/co-supervisions: 2, 1/2</p>	
Thesis co-director	

¹ In EGAAL Doctoral School, if only one scientist in thesis supervision = 100% of supervision rate; if 2 people involved in thesis supervision = from 50% to 70% of supervision rate for the director; if 3 people involved in thesis supervision = 40% / 30% / 30% of supervision rate distribution among supervisors.

Surname, first name:

Position:

Obtained date of the HDR (Habilitation thesis to supervise research):

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Thesis co-supervisor 1 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Thesis co-supervisor 2 (if applicable)

Surname, first name:

Position:

Habilitation thesis to supervise research yes no If yes, date diploma received:

Employer:

Doctoral school affiliation:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Private partner (if CIFRE funding, private funding,...)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

International partner (if Cotutelle thesis)

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

Devenir des anciens doctorants du directeur et co-directeur(s)/co-encadrant(s) de thèse (depuis 5 ans)

Compléter les informations suivantes pour chaque ancien doctorant

Nom, prénom : Zhao, Xue

Date de début et de fin de thèse : Septembre 2016-Décembre 2020

Direction de thèse : Tirichine Leila

Emploi actuel, lieu : Assistant Professor, Chine

Contrat (post-doc, CDD, CDI) : CDI

Liste des publications issues de ce travail de thèse :

1- **Xue Zhao**, Achal Rastogi, Anne Flore Deton Cabanillas, and Leila Tirichine: H3K27me3 natural variation selectively marks genes predicted to be important for differentiation in unicellular algae. **New Phytol.** **2021** Mar;**229(6):3208-3220**.

2- **Xue Zhao**, Anne Flore Deton Cabanilla et al., **Frontiers in Plant Science** **2020**

3- **Xue Zhao**, **Antoine Huguin**, Timothée Chaumier, and Leila Tirichine : Epigenetic Control of Diatom Genomes: An Overview from In Silico Characterization to Functional Studies. **Book chapter in The Molecular Life of Diatoms, Springer Nature Switzerland AG 2021**.

4- Ait-Mohamed O, Novák Vanclová AMG, Joli N, Liang Y, **Zhao X**, Genovesio A, Tirichine L, Bowler C, Dorrell RG PhaeoNet: A Holistic RNAseq-Based Portrait of Transcriptional Coordination in the Model Diatom *Phaeodactylum tricornutum*. **Front Plant Sci.** **2020** Oct 16;11:590949

Nom, prénom : Huguin, Antoine

Date de début et de fin de thèse : Septembre 2016-Aout 2019

Direction de thèse : Tirichine Leila

Emploi actuel, lieu : Post doc IBPC Paris

Contrat (post-doc, CDD, CDI) : contrat post doc

Liste des publications issues de ce travail de thèse :

Huguin A, Rastogi A, Bowler C, Tirichine L. Genome-wide analysis of allele-specific expression of genes in the model diatom *Phaeodactylum tricornutum*. **Sci Rep.** **2021** Feb 3;11(1):2954

Antoine Huguin, Ouardia Ait Mohamed, Feng Yang, Chris Bowler, Auguste Genovesio, Agnès Groisillier, Fabio Rocha Jimenez Vieira, Leila Tirichine : Evolutionary analysis of DNA methyltransferases in microeukaryotes: Insights from the model diatom *Phaeodactylum tricornutum*. *In press. NAR*

Xue Zhao, **Antoine Huguin**, Timothée Chaumier, and Leila Tirichine : Epigenetic Control of Diatom Genomes: An Overview from In Silico Characterization to Functional Studies. **Book chapter in The Molecular Life of Diatoms, Springer Nature Switzerland AG 2021**.

Five main recent publications of the supervisors on thesis subject:

THESIS FUNDING

Origin(s) of the thesis funding: Application to ADEME and Région Pays de la Loire for half doctoral fellowship each

Gross monthly salary: 3 657,41 €

Thesis funding state : Non acquired

Funding beginning date/Funding ending date: September 2022-August 2025

Date: 12/04/2022

Name, signature of unit director:

Simier Philippe



Name, signature of team director:

Tirichine Leila



Name, signature of thesis project director:

Tirichine Leila

