Expected results: This will be the first investigation of the interference of bacteria with microalga-virus population dynamics. Whether we will also test whether the evolution of resistance occurs faster (or slower) upon co-culture with bacteria.

Approach: Population dynamics of Microalgae-virus and Microalgae-bacteria-virus microcosms will be monitored for 3 months using cell cytometry counts and plaque assays (virus). We will test:

1. What is the genomic make-up of the Ostreococcus' bacterial partners?
   - The bacterial partners have been recently isolated from Ostreococcus cultures and have been submitted to the Banyuls culture collection (WDCM91). We have preliminary obtained monitoring of cell abundance of Ostreococcus in co-culture with a bacterial community via flow cytometry. These data show that co-culture of Ostreococcus with these bacteria enables to maintain Ostreococcus microalgae without exogenous addition of any nutrients for more than 12 months, while axenic Ostreococcus cultures die within 2 months, if not re-inoculated in fresh media.
   - Here, we plan to take advantage of these original biological models to study long-term Ostreococcus-bacteria-virus interactions.
   - Specifically, we want to address these 3 objectives:

   **O1- What is the genomic make-up of the Ostreococcus’ bacterial partners?**
   - Approach: Long term co-cultures of Ostreococcus and a commensal bacterial community maintained for more than one year at a stable microalga:bacteria ratio in triplicates will be sampled to process to DNA extraction and sequencing by Illumina. Multimetagenome assembly (9) will be followed by annotation, and comparative genomics of bacterial partners.
   - Expected results: Complete genome sequence, and relative abundance of novel marine bacterial strains. Identification of candidate partners.

   **O2- What are the responses of the microalga to the co-culture conditions?**
   - Approach: From the previous experiment, we will perform RNA sequencing as previously described (8) and metabolomics analysis using Ultra-High Performance Liquid Chromatography coupled with an Orbitrap mass spectrometer. We will use state of the art comparative transcriptomics and metabolomics analysis (10) to detect significative differences in gene expression and metabolites in axenic Ostreococcus versus in co-culture conditions.
   - Expected results: Differentially expressed genes and metabolomic landscapes in co-culture versus axenic conditions in both the microalga and the bacteria, identification of over-expressed and under-expressed metabolic pathways. These results will also provide fundamental insights into the biochemical interactions between the microalga and the bacteria.

   **O3- What are the effect of the bacteria on the Microalgae-virus dynamics?**
   - Approach: Population dynamics of Microalgae-virus and Microalgae-bacteria-virus microcosms will be monitored for 3 months using cell cytometry counts and plaque assays (virus). We will test:
     (1) whether the dynamics of the number of viral particles changes with and without bacteria (adsorption accounted for, see 3 below),
     (2) we will also test whether the evolution of resistance occurs faster (or slower) upon co-culture with bacteria.
     (3) whether the adsorption of viruses onto the microalga is modified in presence of bacteria, using a recently developed protocol (11).
   - Expected results: This will be the first investigation of the interference of bacteria with microalga-virus population dynamics. Whether
positive or negative interactions are detected, this result will impact the modeling of the flow of carbon within microbial planktonic communities.

* References available in extended version

**Stratégie de publication:**
First publication:
Genomic basis of long-term stability of coexistence Ostreococcus and heterotrophic bacteria.
Second publication:
Metabolic cross-talk in a phototroph-heterotroph long-term stability experiment.
Third publication:
Bacterial interference on Microalgal-Virus interactions.

**Réorientation possible du sujet si échecs:**
Because of the preliminary results obtained on bacteria-Ostreococcus coexistence in three microcosms maintained without any external input for more than 12 months, there is no risk that O1 and O2 will fail. However, there is a risk that we will not be able to detect any interference of the bacteria on the microalga-virus interactions. In this case, we may try to publish this negative result, or re-orientate O3 either (i) into an environmental metagenomic survey of bacteria-microalga co-occurrence in the TARA ocean metagenomic dataset or (ii) into an experimental investigation of the metabolic complementarity of the bacterial partners identified in Ostreococcus with other marine microalga species outside the Ostreococcus genera.

**Faisabilité sur 3 ans (échéancier):**
The analysis of the microcosm is planned to be completed within the first year and in the basis of objectives O1 and O2. The microcosm experiment including bacteria, microalga and virus will be started the last semester of the first year. These co-culture experiments do not require a lot of man-time, as the cultures can be sampled twice a week and cryopreserved at -80°C for a global analysis at the end of each month. The bioinformatic tools necessary to the assembly and comparative genomics analysis are routinely deployed in the supervisors’ teams, so that the student will benefit from an onsite ‘hands-on’ training.

**Profil du candidat recherché:**
Microbiologist with a strong interest in bioinformatics and biostatistics.