Proposition de stage

Parcours Master 2 « Microbiologie, Environnement, Santé »

1. **Laboratoire / Entreprise d'accueil :**

Intitulé : laboratoire d'oceanographie microbienne
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2. **Description du stage** (2 pages maximum)**:**

Titre: The use of genetic tools in order to understand how heterotrophic bacteria can cope under Fe-limitation.

Sub Titre: The combined use of the glyoxylate shunt and light under Fe-limitation in marine heterotrophic bacteria.

Mots clés: Fe-limitation, heterotrophic bacteria, genetic tools, gene knockout, bioreporters

Contexte et objectifs généraux:

Fe-limitation within the ocean

Iron (Fe) is an essential element for marine microbial growth but is present in trace amounts (<0.1 nM) in surface waters of the ocean. Little is known about how heterotrophic bacteria can cope under such low Fe-conditions which particularly impacts ATP production as Fe is an essential co-factor of enzymes involved in the electron-transport chain as well as the tricarboxylic acid (TCA) cycle (Tortell, 1996). Fe-limitation can therefore drastically reduce both bacterial growth and respiration, consequently affecting the efficiency of organic carbon remineralization. Heterotrophic bacteria, however, can possess various strategies to cope with Fe-limitation. The objective of this master thesis, is to use a combination of genetic tools (such as the use of knockout mutants and bioreporters) in order to gain further insight into the various strategies heterotrophic bacteria may use under Fe-limitation.

Genetic Tools

The use of genetic tools is key in elucidating such strategies. The construction of a bioreporter can allow us to track and measure gene expression in a non-invasive way over time while gene knockouts further identify the phenotype of the cell and whether the presence or absence of a gene can indeed provide a bacterium with an advantage under Fe-limiting conditions.

The Glyoxylate Shunt

Recently, these genetic approaches were used to elucidate one such strategy, namely the upregulation of the glyoxylate shunt under Fe-limitation (Koedooder et al., 2018 under review). Bacteria can redirect their metabolism away from the classic TCA cycle and towards the glyoxylate shunt which bypasses certain steps such as the production of carbon dioxide (CO_2) and the formation of NADH, thereby reducing the use and dependency of an otherwise inefficient electron transport chain (Kornberg, 1966; Kornberg and Krebs, 1957).

The addition of light: proteorhodopsin

Interestingly, the glyoxylate shunt has also been shown to be induced in the addition of light due to the presence of proteorhodopsin (Palovaara et al., 2014). Proteorhodopsin is a light induced hydrogen pump which may be used to produce ATP or aid in the uptake of Fe within the cell. Therefore, the inclusion of light is crucial towards further understanding the ability of heterotrophic bacteria to remodel their metabolism under Fe-limitation.

Projet de stage:

In this master thesis, the student will use several genetic tools (gene knockouts and bioreporters) to test whether the presence of proteorhodopsin and light can impact the glyoxylate shunt under Fe-limitation. The genetic tools needed in order to conduct the work have already been constructed. The student will learn how to conduct bacterial culture work under trace metal clean conditions and learn general skills required for the growth and maintenance of these cultures. Preliminary experiments will be constructed in order to verify the induction of the glyoxylate shunt under Fe-limitation. Students will obtain several different data including bacterial growth curves, derive bacterial growth and respiration rates from O_2 measurements, measure gene expression over time, and ATP measurements. The student is expected to continue these experiments in order to include the role of light (and the use of proteorhodopsin). Data analysis will include the use of basic R-skills in order to present coherent graphs and their statistical analysis.

Les objectifs de ce stage M2 sont :

January - Introduction to the topic and general lab-work
Students would learn to work with genetic tools under trace metal clean conditions.
Students will learn general skills requiring the growth and maintenance of bacterial cultures.

February – Preliminary Experiments
Students will learn to conduct a set of preliminary experiments in order to become sufficiently …. In order to construct bacterial growth curves, gene expression, respiration rates and ATP measurements. Students will further become accustomed to the topic and their requirements.

March – Experiments
Students will conduct the first set of experiments in order to answer the following scientific questions and their analysis. Basic skills in R (skills including basic graphs and statistical analysis)

April – Experiments
Students will continue experiments (independently) in order to further investigate the following scientific questions.

May – Writing the manuscript
Students will start to write the manuscript and prepare for defence.

June – Submission and Defence

***Bibliographie** :*

1. Palovaara, J., Akram, N., Baltar, F., Bunse, C., Forsberg, J., and Pedrós-alió, C. (2014). Stimulation of growth by proteorhodopsin phototrophy involves regulation of central metabolic pathways in marine planktonic bacteria. PNAS, e3650–e3658. doi:10.1073/pnas.1402617111.Pinhassi et al., 2016
2. Pinhassi, J., DeLong, E. F., Béjà, O., González, J. M., and Pedrós-Alió, C. (2016). Marine Bacterial and Archaeal Ion-Pumping Rhodopsins: Genetic Diversity, Physiology, and Ecology. Microbiol. Mol. Biol. Rev. 80, 929–954. doi:10.1128/MMBR.00003-16.

3. Tortell, P. D., Maldonado, M. T., Granger, J., and Price, N. M. (1999). Marine bacteria and biogeochemical cycling of iron in the oceans. FEMS Microbiol. Ecol.
4. Tortell, P. D., Maldonado, M. T., and Price, N. M. (1996). The role of heterotrophic bacteria in iron-limited ocean ecosystems. Nature 383, 330–332. Available at: http://dx.doi.org/10.1038/383330a0.
5. Kornberg, H. L. (1966). The role and control of the glyoxylate cycle in Escherichia coli. Biochem. J. 99, 1–11. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5337756 [Accessed May 15, 2018].
6. Martin, J. H., and Fitzwater, S. E. (1988). Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. Nature 331, 341–343. doi:10.1038/331341a0.

Sélection d'autres publications de l'équipe sur le sujet:

1. *Koedooder, C.,* Guéneuguès A., Van Geersdaële R., Vergé V., Bouget F.Y., Labreuche Y., Obernosterer, I., Blain S. (in review). The role of the glyoxylate shunt in the acclimation of iron limitation in marine heterotrophic bacteria. Frontiers in Microbiology.

Ce stage peut-il se poursuivre par une thèse ? :
probablement pas au LOMIC