# Evolution of Nitrogen-fixing Bacteria

Leila Fattel March 24<sup>th</sup>

#### Introduction

Nitrogen-fixation is a process carried out by several bacterial and archaeal species. This process is important due to the limited amounts of nitrogen in soil and can therefore affect crop yield [1]. Plants tend to form long-term symbiotic relationships with nitrogen-fixing bacteria to gain accessibility to nitrogen, while supplying energy resources to the bacteria from photosynthesis in return. This association results in the development of root nodules, where the site of nitrogen-fixation occurs [2].

Nitrogen-fixing bacteria have an enzyme called nitrogenase that catalyzes the process of nitrogen (N<sub>2</sub>) conversion to ammonia (NH<sub>3</sub>), a molecule that can be used by plants, in the following reaction [3]:

$$N_2 + 8H + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

The process of nitrogen-fixation takes place by a metalloenzyme complex [4]. This complex is composed of 2 main subunits: the dinitrogenase and the dinitrogenase reductase[3]. This complex, along with other regulatory proteins, is coded by the *nif* genes, which are found in a diverse group bacterial species.

#### Aim

Since the nitrogen-fixing bacterial species are diverse, and can be distantly related to each other in the bacterial species tree, the aim of this project is to investigate the evolution of one or more *nif* genes in different nitrogen-fixing bacteria (did the genes rise by independent convergent evolution, have the genes been lost in some species while being retained in others, or are the genes obtained through horizontal transfer?).

## **Approach**

I will be constructing phylogenetic trees from select bacterial species based on one or more *nif* genes and comparing them with the bacterial species tree (based on whole-genome). The genes and bacterial genomes will be downloaded from the NCBI database. The trees will be constructed using different methods: maximum parsimony, distance-based, maximum-likelihood, and Bayesian, and the best tree from each method will be selected and compared to the others.

### References

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- 2. Ibáñez, F., Wall, L., & Fabra, A. (2017). Starting points in plant-bacteria nitrogen-fixing symbioses: intercellular invasion of the roots. *Journal of Experimental Botany*, 68(8), 1905-1918.
- 3. Bhattacharjee, R. B., Singh, A., & Mukhopadhyay, S. N. (2008). Use of nitrogen-fixing bacteria as biofertiliser for non-legumes: prospects and challenges. *Applied microbiology and biotechnology*, 80(2), 199-209.
- 4. Franche, C., Lindström, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and soil*, *321*(1-2), 35-59.