**Master project: Assessing potential enzyme promiscuity in a cold-adapted bacterium**

*Description*

It might sound surprising but the Earth is a cold planet. Most of its biosphere is exposed to temperatures below 5 degrees. There is a great diversity of life in these cold environments, most of it microorganisms. Organisms living in cold environments are named psychrophiles, which literally means cold-loving (Margesin and Miteva, 2011). Given that the major part of the Earth is exposed to low temperatures, psychrophiles are an important fraction of Earth's biomass (Siddiqui et al., 2013). The study of psychrophilic species is important because of their abundance and their role in the organic matter turnover. Moreover they host multiple proteins well adapted to cold temperatures, and as a consequence, they have biochemical properties that makes them very useful for industrial processes.

Besides cold temperatures, psychrophiles have to face other extreme conditions such as the abundance of reactive oxygen species (ROS), extreme pH, extreme salinity, low availability of nutrients and low light. Evolution has elegantly selected unique traits that allow psychrophiles to survive in such a challenging conditions. Some examples of strategies that cold-adapted organisms have adopted are: up-regulation of proteins with functions that facilitate growth at low temperature (e.g.: protein synthesis, antioxidant activities, protein folding), increasing the fluidity of the membrane by changing fatty acid composition, synthesizing cold-active enzymes, increasing the number of antioxidant enzymes to detoxify reactive oxygen species or the synthesis of molecules that prevent the formation of ice crystals (Margesin and Miteva, 2011; Lauro et al., 2011). This ability to thrive in extreme conditions also comes at a cost: psychrophiles are irreversibly adapted to these extreme environments, and most of them are not able to grow at mild temperatures (Piette, Struvay and Feller, 2011).

Evolvability is the ability of an organisms to adapt to new challenges. An example of a new challenge could be the ability to degrade a new sugar to use it as a carbon source. But how this happens at the genetic level? How can this new ability arise? It has been shown that some enzymes are promiscuous, that is, under certain conditions they can catalyze reactions in addition to those that they originally catalyze (Tokuriki and Tawkif 2009). In fact, it has been shown experimentally how a new function can evolve by mutations that affect the promiscuous function of a given enzyme but do not alter its native function. This observation suggests that enzyme promiscuity facilitates evolvability (Aharoni et al. 2004).

It is well known that enzyme promiscuity can be driven by temperature (Nobeli, Favia and Thornton 2009). For example, *Thermotoga maritima* is a bacteria with an optimal growth temperature of 82 °C. Its genome encodes a thymidine kinase, which shows a high substrate specificity at its optimal growth temperature. However, when the temperature is decreased to 37 °C, the thymidine kinsase turns promiscuous (Lutz, Lichter, Liu, 2007).

*Goal*

We propose an exploratory project to find, for the first time, instances of enzyme promiscuity in a bacterium isolated from the Antarctic sea. Our goal is to assess the evolvability potential of this bacterium in novel environments. To do so, we will grow the bacteria in Biolog plates, which are 96 well-plates whose wells contain different carbon sources, nitrogen sources, phosphorus and sulfur sources, nutrient supplements, peptide nitrogen sources, osmolytes, pH or chemicals (such as antibiotics). Proteins from cold-adapted bacteria are adapted to low temperatures, and therefore, their protein flexibility (and potentially, promiscuity, because a more flexible protein can putatively bind additional ligands) increases with temperature. By growing the bacteria at their optimal temperature (15 °C) and at higher temperatures (24°C and 28°C), we aim to find Biolog wells that contain sources that support growth only at higher temperatures. These sources are candidates to study promiscuity in more detail.

This project will prepare the ground for a larger project in which we will use experimental laboratory evolution to adapt our model psychrophilic bacteria to the “promiscuous” sources identified using the Biolog plates. By combining experimental evolution with whole genome sequencing we will study the molecular paths to evolvability.

This project is addressed to Biology and Biochemistry students with an interest in evolution and microbiology. In this project you will learn basic microbiology techniques (bacteria cultivation, Biolog assays, growth curves) and you will become familiar with statistical analyses and experimental design. We offer a young and dynamic research environment and a well equipped laboratory at the University of Zurich (Irchel Campus).

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