Laboratory evolution of microbial communities for increased metabolite production

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Evolution and adaptation through natural selection are cornerstone concepts of Biological sciences. The recent advances in the fields of Microbiology and Molecular Biology allowed scientists to introduce evolution in controlled laboratory settings. Adaptive laboratory evolution (ALE) has been successfully applied to better understand the effect of natural selection on individuals, as well as to obtain cells with improved phenotypic characteristics. In the majority of the reported cases, the characteristics that are targeted for improvement are related to biotechnological processes, aiming to create improved microbial strains for industrial applications. However, ALE is limited to growth-associated traits, such as substrate utilization and increased tolerance of compounds that inhibit growth.

The aim of this PhD thesis was to develop novel methodologies that could overcome the major bottleneck of ALE to enable the improvement of non-growth associated traits for non-genetically modified organism (GMO) biotechnological applications. For this reason, small synthetic obligatory mutualistic communities were established. The design of a metabolic cross-feeding relationship between the species in the community couples the production of a target metabolite to the survival and proliferation of the community. Increased concentration of the target metabolite in the environment results in improved community fitness, despite of any potential production cost. Communities consisting of natural vitamin secreting lactic acid bacteria and engineered *Saccharomyces cerevisiae* were successfully evolved for the improved production of two different B group vitamins (riboflavin and folate). The isolated evolved overproducing bacterial strains can be used for the production of food with increased nutritional value.

The results of this work demonstrate that the developed method can increase the precision of laboratory evolution and allow the selective production of fitness-costly metabolites. The phenotypic characteristics of both prokaryotes and eukaryotes could be improved, and the obtained strains hold potential for biotechnological applications, especially when the use of genetically engineered strains is restricted. Apart from the potential biotechnological applications, the designed laboratory evolution strategies can also be exploited to shed light on open questions about the physiology, the ecology and the social life of microbial species and communities.