





## Heterologous proteins production: A comparison between a bacterial and a yeast cell system

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Microorganisms, such as bacteria and yeast, are commonly used for the production of heterologous proteins, which are component of diagnostic, therapeutic tools and industrial enzymatic kits.

Our research focused on producing heterologous proteins from the bacteria *Escherichia coli* and the yeast *Pichia pastoris*. We investigated different experimental approaches tailored to the metabolic and physiological proprieties of each microorganisms.

In the first approach, performed in collaboration with the Enzymology and Protein Engineering laboratory, we produced in *E. coli* three enzyme involved in the design of a "in vitro" synthetic enzymatic cascade able to bio-convert glycerol into metabolites of greater value such as pyruvic or lactic acid.

In the second approach, performed in collaboration with the Nanobiolab laboratory, we produced in *P. pastoris* two recombinant protein: a chimeric construct, consisting in a suicidal enzyme linked to a scFv antibody and a modified ScFv alone. Both proteins are involved in the functionalization of different types of nanoparticles, in order to direct them towards pathological targets, as tumours.

For both projects, we evaluated different aspects of the cellular cultivation. At first, we investigated the physiological response to: different carbon sources, induction strategies, supplementary growth factors and cellular inoculum preparation. On the other hand, we evaluated the response to chemical and physical parameters such as temperature, culture medium buffering and different level of aeration.

The results obtained indicate that it is necessary to develop specific production strategies, considering both the microorganism involved and the protein of interest. We observed that some cultivation approaches resulted similar between prokaryotes and eukaryotes. While, as reported in literature, different recombinant proteins need "ad hoc" production strategies.

