Proteins obtained through microbiological production are widely used as medicines, vaccines, diagnostic test components, in the production of food, washing and cleaning agents, cosmetics as well as in the processing of waste biomass. During the production process, the cells of the microbiological producer are exposed to numerous stress factors, caused by both external conditions and internal bottlenecks, resulting from the redirection of metabolism towards the flux designed and forced by man. High capacity for production of a desired protein during a bioprocess results from many factors, including efficiency of a cellular translational-secretory machinery for protein synthesis, and the resistance of the producer cells to emerging environmental stress factors.

Both highly efficient translational-secretory machinery as well as resistance to the stress factors are complex features that depend on the accurate and coordinated operation of many processes in the cell, for which numerous genes and proteins are responsible. The complex nature of these features makes the attempts to improve them a serious challenge, as it is difficult to unambiguously identify the elements that determine them (individual genes, their combinations or specific processes). Modification of one or several genes involved the complex trait is often compensated by cellular mechanisms of homeostasis maintenance. Therefore, it is assumed that in the case of complex features modification, only global approaches, that allow for the introduction of multidirectional ("massive") changes, are a reasonable solution. Traditionally, such modifications were introduced randomly, which is currently being abandoned due to the lack of control over the course of the process. One of the innovative solutions is to trigger massive changes in the cell functioning through controlled manipulation with transcription factors. Transcription factors (TFs) are intracellular protein elements that govern gene expression in response to stimuli from outside / inside the cell, causing massive changes in the cell functioning. Noteworthy, the resulting response induced by the action of a given TF is coordinated, adequate and targeted, which means that numerous and seemingly unrelated molecular elements (e.g. energy metabolism, functioning of cell membranes and amino acid metabolism) are subjected to modulation, ultimately causing a comprehensive and the most effective response to a given stimulus that a cell can generate.

The primary aim of this project is to indicate TFs contributing to increased resistance of microbial cells to stress factors typically occurring in the biotechnological production processes, and / or enabling increased production of valuable proteins, which can further be used in various areas of human activity. The proposed study will be carried out using *Yarrowia lipolytica* yeast. *Y. lipolytica* is a safe nonconventional yeast species (GRAS status issued by FDA and EFSA) currently widely used in scientific research and in industrial practice, *e.g.* for the production of feed (for horses and cattle), proteins (*e.g.* lipase, protease), citric acid, or natural, calorie-free sweetener (erythritol). Most TFs in *Y. lipolytica* have only been identified recently based on a total secretome scanning using bioinformatics tools, but their molecular function still remains unknown.

It is planned to conduct experimental work using a collection of 125 recombinant *Y. lipolytica* strains in which the genes encoding the newly identified TFs are overexpressed together with easy-to-follow reporter proteins. This library will be subjected to intensive screening studies under various conditions (including stressful ones) in order to indicate the TFs being responsive to a given environmental factor. Designed experimental plan covers initial screen in 78 different variants. The conditions (including. stress factors) were selected on the basis of their known impact on the growth and metabolic activity of *Y. lipolytica* and the other, related species, adequacy to biotechnological production processes, and their known interaction with the protein production efficiency. It is planned to investigate whether manipulation with TFs will facilitate growth and viability maintenance under adverse conditions, *e.g.* reduced oxygen availability, change in nutrients' availability, acidic pH or different temperatures. In-depth studies are planned to be carried out with selected TFs, shown to improve *Y. lipolytica* cells' capacity to produce proteins despite the unfavorable environmental conditions occurrence.

The research will use modern methods allowing for conducting multiple cultivations and analyzes in parallel. The studies will be conducted using innovative techniques and solutions, i.e. GoldenGate modular cloning, CRISPR-Cas9 genome editing, miniaturized, high-throughput cultivations, transformation and biochemical assaying techniques. In addition, characterization of selected TFs' regulomes (genes governed by the TF) will be performed using global-scale omics techniques (RNAseq) and continuous cultures in bioreactors under steady-state conditions.

The project results will allow to acquire new basic knowledge with high potential for practical benefits. Gaining new knowledge about the molecular basis of cell activity, *i.e.* unknown TFs, their function, mechanisms of action and role in developing biotechnologically relevant features is expected to be acquired. Proposition of new genetic engineering strategies, with a significant impact on the basic and applied research, is also foreseen. To the best of the research team's knowledge, no such high-throughput studies on TFs impact on heterologous proteins production in relation to industrially-relevant stress conditions have been conducted to date in *Y. lipolytica* or the other yeast species.