Proteins derived from microbial production are commonly used in different areas of human activity, like medicine, diagnostics, food manufacture and production, household care products, cosmetics, biofuels production, as well as in the processing of waste biomass. Substantial fraction of these proteins are produced using yeast cells, which belong to highly potent protein producers, but are also subjected to numerous limitations. It has been estimated that a given protein excreted outside the cell (for easier collection) can be produced at even 1000-fold lower titers than the theoretical maximum, when not optimized at molecular and/or bioprocessing level. According to another relevant estimation, over 30% of all the proteins (both foreign and native) are processed by the secretory pathway, which directs the proteins outside the cells. The secretory pathway is highly complex, as it involves multitude of genes/proteins and spans cellular compartments. Therefore, it requires an in-depth knowledge on the cell's biology to modify or improve this pathway. Considering that the world market of r-Prots constitutes one of the key branches of current industrial biotechnology, earning billion dollar (USD) revenues each year, studies into biology of the secretory pathway is relevant for both basic and applied research.

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). It has gained significant attention as a platform for recombinant (foreign) secretory (excreted outside the cell) proteins (rs-Prot) production due to multiple advantageous characteristics of its secretory pathway.

In my former research I compared whole-cell gene expression profiles of *Y. lipolytica* producing different rs-Prots. Using a global approach (analyzing expression of all the genes in the genome) and careful manual inspection of data, the study allowed gaining great amounts of new knowledge. It was possible to identify genes and biological processes that are involved in synthesis and secretion of rs-Prots in *Y. lipolytica.* Many of these were found similar to what was observed for the other yeast, so are expected to have similar biological function, but there are still some whose role remains elusive.

In the proposed project, I'd like to continue the studies on the rs-Prots synthesis and secretion in *Y. lipolytica*, using the previously gained knowledge as a guide for genetic engineering strategies. The main objective of the project is to study specific function of selected genes, of confirmed implication in rs-Prot synthesis in *Y. lipolytica* based on the previous global study, and assessing possibility of these genes exploitation factors enhancing rs-Prots synthesis. Selected GOIs potentially play a role in the following cellular processes: Oxidative stress response, Proteolytic degradation, and Cell wall modifications; which was inferred based on their similarity to genes from the other yeast species that are better described than *Y. lipolytica*.

The concept is to determine function of ten GOIs involved in rs-Prot synthesis in *Y. lipolytica* by their overexpression (increasing amounts of active protein product) and deletion (elimination of the protein product from the cell) followed by assessment of the modified cells growth / behavior in laboratory cultures. To combine the GOIs' impact on rs-Prot synthesis, the strains will simultaneously produce rs-Prot that is easy to measure. Two different rs-Prots were chosen to test the GOIs impact on different proteins – one is enzyme and the second – emits fluorescence. Overexpression and deletion will be conducted using modern genetic engineering tools, and the generated strains will be cultivated in small-scale optimized cultures. To accurately describe what global changes were caused by the conducted modifications of GOIs, selected strains will be cultivated in strictly regulated bioreactor cultures, and expression of all the genes in the cell will be conducted. All the equipment, tools and skills required for the project's completion were mastered and are in use in the laboratory.

The project is expected to provide experimental evidence on molecular function of uncharacterized GOIs and their impact on rs-Prots production in *Y. lipolytica*. Moreover, it is foreseen that as a result of the project completion, genes associated with the GOIs will be indicated and described, based on global gene expression studies. Consequently, proposition of new strategies for enhancing secretory protein production in yeast is expected.

The project will provide new, exciting basic knowledge with high potential for practical benefits. It is expected that the results will be published in international journals and presented during international conferences.