Popular science abstract of the project

At the present time, when the world's demand for energy is growing and the resources of crude oil and natural gas are running out, the demand for alternative energy sources is growing. The use of oilseeds for the production of biodiesel turned out to be economically unjustified due to the crisis of the food economy in many regions of the world, as well as for economic reasons. In order to meet the demand for biofuels, alternative methods of their production have proven promising, such as using lignocellulosic biomass or microorganisms, which are characterized by their amazing ability to accumulate intracellular lipids. There are microorganisms that are able to accumulate up to 80% of lipids on a dry weight basis. One of the mentioned microorganisms are the yeast *Yarrowia lipolytica*. This species, also due to the sequenced genome and a wide range of genetic engineering tools, has gained wide interest of scientists and has become a model organism for the process of lipid biosynthesis. However, the dynamic development of biotechnology has led to the discovery that these yeasts can also release large amounts of polyhydroxy alcohols into the environment, which, thanks to their chemical properties, can replace sucrose in food products. These amazing microorganisms are part of a broader group of microbes called the *Yarrowia* clade, which currently comprises 15 different yeast species. The huge interest in these microorganisms allowed to prove that among the species forming the clade, there are those whose ability to accumulate lipids or secrete polyhydroxy alcohols is definitely higher than for *Y. lipolytica*. It also turned out that this yeast is able to effectively use waste compounds, such as crude glycerol or volatile fatty acids (VFAs), which are found in high concentrations in post-fermentation mixtures of food industry waste or municipal waste. *Yarrowia keelungensis* or *Yarrowia phangngaensis* are characterized by a much better assimilation of these substrates than the *Y. lipolytica* species. In addition, *Yarrowia yakushimensis*, as the only representative of the clade, is characterized not only by a higher affinity to acetate, which it uses before propionate or butyrate, but it has also the only hexokinase lacking the 37-amino acid loop present in hexokinases from other species of the *Yarrowia* clade. Due to the fact that hexokinase belongs to the so-called moonlighting proteins, i.e. apart from kinase activity, it also participates in the regulation of gene expression, it seems important to analyze the regulation of gene expression in the *Y. yakushimensis*. In addition, the analysis of the transcriptome in the other two mentioned species will be the starting point for improving substrate utilization by the clade representatives. The characterization of glycerol and VFA metabolism as well as the lipid biosynthetic pathway by using an automated colony manipulation system is also an important part of the proposal. The analysis of several tens of thousands of transformants will allow the selection of new, so far uncharacterized enzymes/transporters/transcription factors that will help to understand the functioning of the metabolic pathways mentioned above in selected species from the *Yarrowia* clade. In addition, an extremely important aspect proposed in the project is the use of the enormous protein secretion capacity of yeast from the *Yarrowia* clade, for the production of heterologous proteins, especially those with the activity of surfactants. These proteins will be able to replace chemically synthesized surfactants used e.g. in mining industry. However, having different physicochemical and sometimes biological properties, surfactants are used in various forms and perform various functions in the production of many products used in households, orchards and agricultural fields, in the automotive industry, construction, road construction, as well as in the cosmetic and pharmaceutical industries . Each of the listed industries is looking for alternative, biodegradable compounds whose properties would allow the development of environmentally friendly technologies. The solution proposed in the project will allow to assess the possibility of simultaneous biosynthesis of lipids and proteins with surfactant activity from waste substrates obtained from other industries.