

For thousands of years, humanity has explored the natural environment to discover and obtain valuable substances for its own various applications. Such explorations have led to the discovery and development of a method for producing many medicines, health-promoting dietary supplements, dyes, flavors, and fragrances. Many of the products known today, produced by chemical synthesis methods, were inspired by substances originally isolated from the natural environment.

Along with the growth of the human population and the desire to raise the standard of living, the exploitation of the natural environment has gained an unfavorable dimension. Obtaining chemical compounds by extraction from natural materials, or environmental pollution by components used in chemical synthesis processes, contribute to the devastation of the natural environment. One of the possibilities for reducing the adverse impact of human activity on the natural environment is to transform these methods into biotechnological processes based on microorganisms.

As it turns out, the metabolism of microorganisms is so plentiful, diverse, and flexible that it is possible to develop biosynthesis processes of compounds, originally discovered as specific to plants or animals, in the cells of microorganisms. In this way, without the need to further exploit the natural environment, in processes conducted under mild, environmentally friendly conditions it is possible to obtain a whole range of chemical compounds desired in various areas of human activity.

One of such high-value chemical compounds is (-)-Ambroxide. It is the main fragrance component of ambergris – a substance formed and excreted from the digestive tract of the sperm whale. The scent of ambergris is described as subdued, warm, and reminiscent of the aroma of earth or sandalwood, with notes of moss, seaweed, tobacco, wood, and earthy accents. Yet, the applications of (-)-Ambroxide are not limited to creating the scent of luxury perfumes. What is more, (-)-Ambroxide belongs to a group of extremely valuable chemical compounds called terpenoids, which include, among others, carotenoids, chlorophylls, steroids, etc., known compounds used in medicine, but also responsible for the taste and smell of many plant foods. These compounds are formed as products of branching of the same metabolic pathway.

In this project, we propose to construct safe yeast strains capable of efficient production of terpenoid compounds, using (-)-Ambroxide as an example. We plan to use two yeast species – *Saccharomyces cerevisiae* (a well-known and widely used baker's yeast species), for which, a part of the target metabolic pathway has been developed by the Chinese team (reaching a specific intermediate), and *Yarrowia lipolytica* – a yeast species, characterized by high propensity to grow efficiently from lipid substrates. In this project, we plan to apply a complementary approach and use the knowledge and experience already acquired in establishing the pathway of intermediate synthesis in *S. cerevisiae*, and transfer the ability to grow efficiently on lipids from *Y. lipolytica* to *S. cerevisiae*. Thus, it will be possible to produce a valuable compound from cheap waste substrates (ultimately - from waste lipids).

During the construction of strains, we plan to make discoveries and gain new knowledge. For example, enzymes that effectively catalyze the conversion of the intermediate to (-)-Ambroxide are not currently known – we plan to identify them in other microorganisms and transfer them to our target yeasts to 'assemble' the entire metabolic pathway in them. We also plan to check whether enclosing the synthesis pathway in specific cell compartments (organelles) will benefit the efficiency of (-)-Ambroxide production from lipids. We anticipate that simply introducing the metabolic pathway of (-)-Ambroxide synthesis from lipids may not be sufficient and it will be needed to optimize the pathway's operation within the structures of yeast metabolism. This will involve a detailed study of yeast metabolism with the 'new' pathway. We also want to develop a composition of the culture medium that will allow for increased synthesis of various terpenoids, including (-)-Ambroxide, using beta-carotene as an example.

The project aims to construct safe yeast strains capable of producing (-)-Ambroxide from waste lipids. Research groups from Poland and China plan to prepare joint publications and conference presentations, and apply for patent protection of their inventions. We expect that this project will contribute to strengthening scientific cooperation between teams and countries, and will constitute a foundation for further research activities.