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Pollution of the planet with plastic waste has become increasingly apparent in recent years. The increased production and use of such materials in a wide range of industries, combined with an underdeveloped waste management system, has resulted in an increasing number of ecosystems being harmed by the accumulation of this litter. One of the most common plastics in the world is poly(ethylene terephthalate) (PET), which is considered a non-biodegradable polymer with outstanding physical and chemical properties. Currently, scientists are undertaking intensive research aiming at solving this feasible and escalating problem and to date, the results were the identification of enzymes from the hydrolase class, such as cutinases, lipases and PETase, as capable of hydrolyzing the ester bonds present in polyesters (P1|). The current work focuses on examining the ability of modified *Yarrowia lipolytica* yeast to degrade plastics and aims to introduce a novel method for degrading polyesters directly in microbial culture.

The increased production and use of plastics in recent years has resulted in their significant accumulation in the environment, which negatively affects the entire ecosystem. One of the world's most common plastic material is poly(ethylene terephthalate) (PET) is one of the world's most widely used plastic material with wide range of usage i.e. packaging, construction and automotive industry. To the date, the enzymes from hydrolase class such as cutinases, lipases and PETase were classified as capable to hydrolyze ester bonds present in polyesters.

First, the research was focused on aliphatic plastic degradation, where *Y. lipolytica* yeast strain extracellularly producing cutinases from *F. solani* and *T. reesei* with co-expression with native *Y. lipolytica* lipase (P2). The work within this scope focused on investigation the enzymatic activity of the enzymes present in the culture's supernatant, the capacity to form clear zones on the emulsified polyester substrate medium, the quantitative assay of the amount of released ε-caprolactone during decomposition process, and the mass loss of biodegradable plastic films. In this study, we have determined the best candidate for further work with more challenging plastic material is a strain producing cutinase from *F. solani*,

employed also for biodegradation of PET (P3). Considering that PETase from *Ideonella sakaiensis* does not exhibit the ability to hydrolyze ester bonds present in aliphatic polyesters, the strain producing this enzyme, along with the strain selected in the first stage of the studies, was used to examine degradation of PET material (P4). The investigation of the capacity to hydrolyze this plastic were performed directly in the culture of the above-mentioned *Y. lipolytica* engineered strains. In addition, we have tested the supplementation influence on the degradation efficiency with the use of various salts and olive oil at different concentrations. Plastic degradation capacity by *Y. lipolytica* strains was determined based on the amount of released PET hydrolysis products such as terephthalic acid (TPA), mono-(2-hydroxyethyl) terephthalic acid (MHET) and with the use of ultraperformance liquid chromatography (UPLC). Furthermore, we have also investigated the ability to assimilate terminal PET degradation products by *Y. lipolytica* and compared the ability to hydrolyze MHET by modified strains producing cutinase from *F. solani* and PETase from *I. sakaiensis*. Finally, the yeast culture with PET film was carried out and the structure of the film was verified by scanning electron microscopy.

The research conducted in this thesis indicates that *Y. lipolytica* is a highly suitable candidate which may be used as a host organism for the extracellular production of enzymes hydrolyzing polyesters. Due to the stabilization of the pH of the culture medium after 72 h of culture within the range of optimal environmental conditions for both enzymes used (pH 8.0-8.5), plastic degradation process can be accomplished directly in the microbial culture. Additionally, in our study we have demonstrated that *Y. lipolytica* is able to assimilate ethylene glycol (EG), which, along with TPA, is the final product of the hydrolysis of this polymer.

Keywords: PETase; cutinase; Lipase; Poly-ε-caprolactone; PCL; *Yarrowia lipolytica*; Poly(ethylene terephthalate); PET degradation; Plastic waste; Genetic engineering