

Nature created many efficient ethanol producers from glucose and sucrose. Annual revenue of fuel ethanol and alcoholic beverages (wine, beer, strong alcoholic beverages etc.) produced during yeast fermentation exceeds 1,500 billion US dollars which is comparable with total cost of metallurgy products. Glucose is the most abundant sugar in the universe whereas two pentoses, D-xylose and L-arabinose, are the second and third most abundant sugars. In contrast to glucose, these sugars do not meet in nature in a free state, therefore, relatively few microorganisms can metabolize and even less can ferment them to ethanol. However, future biofuel industry based on use lignocellulosic feedstock needs the yeast strains capable of effective fermentation of at least three sugars: glucose, xylose and L-arabinose. As a rule, amounts of accumulated ethanol from pentoses are extremely low, and the reasons of that have not been elucidated. We study alcoholic fermentation of the most abundant pentose, xylose, in the natural xylose metabolizing thermotolerant yeast *Ogataea polymorpha*. Wild-type strains *O. polymorpha* vigorously grow on xylose and glucose but accumulate negligible amounts of ethanol from pentose whereas glucose is fermented quite effectively. Growth on L-arabinose is poor. Using methods of classical selection and metabolic engineering, much more advanced ethanol producers from xylose were isolated. Most efficient approaches were based on knock out of genes *CAT8* and *ATG13* coding for transcription activator and autophagy-initiating protein, respectively, and on overexpression of the peroxisomal enzymes. Further improvement of available strains depends on our understanding the regulation of xylose metabolism and fermentation, especially at genetic level. In current proposal, we plan to identify the new genes involved in regulation of xylose and L-arabinose fermentation. Among them, the master regulatory gene which determines the carbon flux from pentoses to ethanol hopes to be identified. Mechanisms of action of the identified regulatory genes will be elucidated using modern methods of yeast molecular genetics. This will permit the drawing the overall picture of gene regulation responsible for xylose and L-arabinose fermentation. The possible role of environmentally friendly phosphoketolase pathway of xylose utilization in *O. polymorpha* also will be studied. Favorable for ethanol production changes in regulatory genes are planned to be combined in one genome resulted in construction of the advanced ethanol producers from pentose sugars.