STUDY OF TRANSHYDROGENASE SYSTEMS FEATURES IN THE MUTANTS OF THE YEAST *PACHYSOLEN TANNOPHILUS* FOR THE PRODUCTION OF ETHANOL AND XYLITOL FROM AGRICULTURAL WASTES

BSE 2020

Olga Bolotnikova[1,2], Julia Bazarnova[2], Ekaterina Aronova [2], Tatiana Bolotnikova[2]

[1] Petrozavodsk State University, Lenina Str. 33, Petrozavodsk, 185910, Republic of Karelia, Russia

[2] Peter the Great St. Petersburg Polytechnic University, Polytechnicheskaya Str. 29, Saint Petersburg, 195251, Russia

The different catabolic enzymes of D-xylose, an important structural component of agricultural wastes, were studied in the mutant strains of the xylose-assimilating yeast *Pachysolen tannophilus*. The evaluation of activity and cofactor specificity of xylose reductase (XR) and xylitol dehydrogenase (XD), s well as activities of 1-glycerophosphate dehydrogenase (GDP), lactate dehydrogenase (LD), total malate dehydrogenase (MD) and cytochrome c oxidase (CO) revealed the metabolic characteristics of the yeast, which could ensure selective ethanol or xylitol production.

Methods

It has been experimentally proved that the xylose-assimilating yeasts *P.tannophilus* have the unique ability to produce comparable amounts of xylitol and ethanol during microaerobic fermentation of D-xylose. This practical result makes possible studying the conditions conducive to the formation of each target product on the example of the same model object. Therefore, based on the *P.tannophilus* -Y-1532 (Museum of Microorganisms, All-Russian Research Institute of Hydrolysis, St. Petersburg), the haploid strain *P.tannophilus* 22-Y-1532 was isolated. Its two-day-old cells were exposed to 1-methyl-3-nitro-1'-nitrosoguanidine under experimental conditions. Thus, were selected 3 mutants, that produced xylitol (No. 664) or ethanol (No. 390, No. 442) from D-xylose. The inoculum for fermentation was obtained by growing yeast on mediums with 2% D-glucose and 2% D-xylose as sole carbon source (230 rpm, 30±20°C,18-24 h). The yeast biomass 6.0±0.5 g d w l^{-1} was used for the microaerobic fermentation of 2.0% D-xylose (100 ml) at 250 ml round-bottom (100 rpm, 30±2^{0}C, 24 h). The concentration of yeast biomass after fermentation was analyzed spectrophotometrically. The concentration of reducing substances (RB) was determined with Fehling's reagent. Ethanol and xylitol were determined by gas chromatography (Vista 600, “Varian”, USA). The concentration of total protein was determined by the Lowry method. Cell-free yeast extracts used to determine the activity of D-xylose catabolism enzymes. The total and specific activities of XR and XD were evaluated by monitoring the concentrations of NAD(P)×H/NAD(P)$^+$ redox pairs at 340 nm. A similar monitoring of the concentrations of the NAD×H/NAD$^+$ pair was used for spectrophotometric determination of LD activity, total MD, and GDP. The activity of CO was determined after its recovery with ascorbic acid according to the procedure.

Results

The activity of XR and XD in the mutant cells turned out to be lower than the control values. The lowest XR and XD activity was observed in mutant No. 664, which showed a low degree (56.0%) and a rate of utilization of D-xylose (0.078 g g^{-1} h^{-1}) . The XR of xylitol producing mutant had a predominant affinity for NADP×H. The highest experimental values of XR and XD activity were found in ethanol-producing mutants that used 86.0% (strain No. 390) or 78.6% (strain No. 442). D-xylose. The rate of its consumption under microaerobic conditions increased to 0.119 g g^{-1} h^{-1} and 0.109 g g^{-1} h^{-1}, respectively. The pronounced double NAD×H/NADP×H specificity of XR was a common feature of these mutant strains (Fig).

The activities of GPD, total MD, CO and LD of the mutants in most cases were lower than the control *P.tannophilus*. Thus, its activities of the ethanol-producing mutants were significantly different. The strain No. 390 was characterized by a more than two-fold increase of LD activity against the background of significant decrease of GPD, total MD and CO activities. On the other side, the mutant No. 442 had activities of all these enzymes, comparable with the dates for the parent strain *P.tannophilus* (Fig).

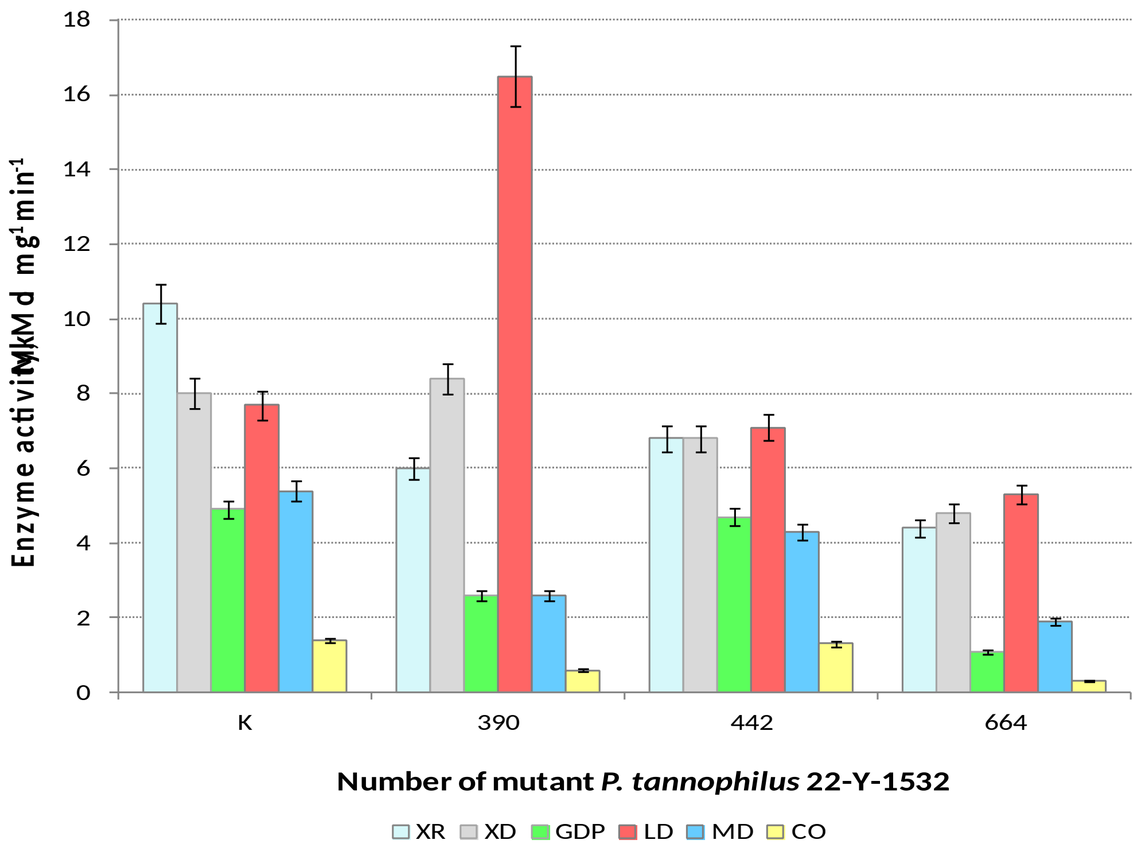

Figure Activity of enzymes involved in the catabolism of D-xylose in mutants of the yeast *P. tannophilus*

K - the parent strain of *P. tannophilus* 22-Y-1532

XR - xylose reductase

XD - xylitol dehydrogenase

GPD - 1-glycerophosphate dehydrogenase

LD - lactate dehydrogenase

MD - total malate dehydrogenase

CO - cytochrome c oxidase

Conclusions

An experimental analysis of the mutants of *P.tannophilus* showed that the production of xylitol and ethanol from D-xylose represents as the rearrangement of whole metabolism of carbohydrates in oxygen deficiency - the final electron and proton acceptor by NAD×H. The increased ratio of NADP$^+$/NAD×H in microaerobiosis is not only the difference in the coenzyme specificity of XR and XD, but also the inhibition of NAD×H/NAD$^+$ dehydrogenases of common sugars catabolism pathways. High production of xylitol may be a manifestation of "the Pasteur effect” for D-xylose, a reserved carbon source in the yeast cells.

The production of ethanol from D-xylose is impossible without flexible regulation of NADP$^+$/NAD×H balance in oxygen deficiency. In addition to the respiratory chains of mitochondria, the xylose-assimilating yeast has at least three to four NAD×H regeneration points. One of them is reduction of D-xylose to xylitol, although the degree of its influence on the balance of NADP$^+$/NAD×H directly depends on the activity and specificity of XR. The participation of pyruvate and dioxiacetonphosphate, as well as the mitochondrial respiratory chains in the regeneration of NAD$^+$, clearly illustrates the close relationship of ethanol formation from D-xylose with yeast growth. A likely explanation for this phenomenon is the greater sensitivity of the xylose-assimilating yeast to ethanol in compared to *S.cerevisiae.* Therefore, studying the genetic system of of *P.tannophilus* becomes an important prerequisite for determining the biochemical steps that restrict the production of ethanol from D-xylose. It will help determine the strategy for genetic construction of yeast strains with high yield of ethanol and xylitol on the base of variety agricultural wastes.

ACKNOWLEDGMENT

This work was supported by the grant No 18-44-100001 from Russian Foundation for Basic Research (RFBR).