XVI Congreso Nacional de Biotecnología y Bioingeniería
21 al 26 de Junio de 2015 Guadalajara, Jalisco, México.
Guadalajara

EHANCEMENT OF ERYTHRITOL PRODUCTION FROM YARROWIA LIPOLYITCA BY METABOLIC ENGINEERING

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Metabolic engineering, erythritol,Yarrowia lipolytica

Introduction. Erythritol is a 4-carbon polyol used in the food industry for its sweetening properties: it is 70% as sweet as sucrose, it is almost non-caloric, and it does not affect glycemia. Currently, erythritol is mainly produced by fermentation using osmophilic microorganisms. One of these organisms is the non-conventional yeast Yarrowia lipolytica. Its main characteristics are the ability to degrade hydrophobic substrates, a great tolerance to salinity and pH and the ability to produce high amounts of proteins and metabolites of interest.
The aim of the present work is to optimize erythritol production from glycerol in *Y. lipolytica* by using a metabolic engineering approach.
Methods. . Strain used was Yarrowia lipolytica JMY2900. Cultures were carried in 250ml shake flasks containing 50ml production médium as described in Tomaszewska et al. [1], at 28°C and 190RPM for 300hours. Erythritol production was measured using RID-HPLC.

Results. Key genes involved in erythritol biosynthesis (figure 1) were cloned and overexpressed in *Y. lipolytica*, under control of the strong constitutive promoter pTEF. Different strains were obtained, each one overexpressing one of those key genes.
Shake-flask cultures were carried in glycerol medium for 10 days. Results showed a significantly higher erythritol productivity for the strain overexpressing glycerol kinase gene (GUT1) while other strains did not differ significantly from the control (figure 2). This indicates that glycerol assimilation might be a determining step in erythritol production.

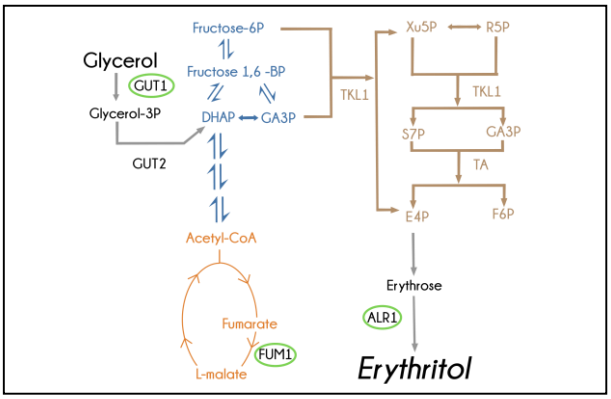

Fig. 1. Erythritol biosynthesis pathway. Overexpressed genes are circled

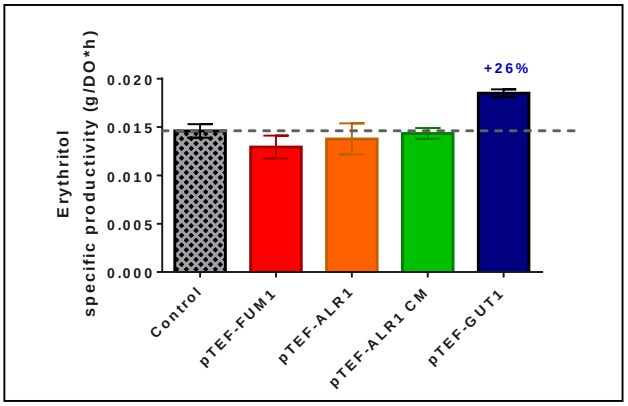

Fig. 2. Erythritol productivity values of overexpressing *Y. lipolytica* strains

Conclusions. These preliminary results show the potential benefits of a metabolic engineering approach to increase erythritol synthesis in *Y. lipolytica*. The next steps will be to construct strains overexpressing a combination of these key genes in order to find potential synergetic eects. This might lead to the creation of an overproducing strain, or perhaps more interestingly, a strain able to produce erythritol in any medium, without osmotic or pH requirements.

Acknowledgements. This work was funded by the Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA)

References.

1. Tomaszewska L., Rywinska A., Gladowski W. (2012). *J Ind Microbiol Biotechnol*. 39: 1333–1343.