The implications of nitrogen on the fermentative growth extension of *Saccharomyces cerevisiae* by isoproturon

M. Candeias[1,2], I. Alves-Pereira[1,2] and **R. Ferreira[1,2]**

[1] Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM-CTA), Universidade de Évora, Núcleo da Mitra, Apartado 94, 7002-774 Évora, Portugal
[2] Departamento de Química, Escola de Ciência e Tecnologia, Universidade de Évora, Rua Romão Ramalho 59, 7002-554 Évora, Portugal

The peptone is used to provide nitrogen to *Saccharomyces cerevisiae*, which is an essential element for growth. In nature and in industrial musts, essential nutrients for yeast growth are available in complex and variable flux. Some of these compounds are herbicides, where some specific microorganisms are capable to mineralize or degrade into more basic and less harmful compounds to the environmental. The isoproturon (IPU), a phenylurea used as an herbicide, is a compound very difficult to degrade in soils and aquifers, reaching levels considered toxic by European legislation, contributing to both surface and ground water pollution, and it may be also involved in the triggering of serious illnesses. So, it's urgent to discover biological models to contribute to degrade or eliminate phenylureas in situations of accidental or systematic contamination.

The main target of this study was to evaluate the influence of nitrogen to extend the fermentative phase of *S. cerevisiae* by IPU, using the wild-type strain UE-ME$_3$ deposited in the collection of Enology Laboratory of University of Évora, Portugal.

Cells at mid-exponential phase were inoculated in presence of 100µM IPU in YEPD or YED medium and incubated during 72 h with orbital stirring, at 28 ºC. Samples from each treatment were used to obtain OD, cfu, dry weight and to prepare post-12000 *g* supernatant for determination of protein [1], glutathione (GSH,GSSG) [2] and malondialdehyde (MDA) [3] contents, and cell capacity to scavenge free radicals by the 2,2-diphenyl-1-picryl-hidrazil (DPPH) [4] method, as well as, enzyme activities catalase T (CAT T) [5], glutathione reductase (GR) [6], glutathione peroxidase (GPx) [7], glucose-6-phosphate dehydrogenase (G6PD) [8], alcohol dehydrogenase (ADH) [9], malate dehydrogenase (MDH2) [10] and lactate dehydrogenase (LDH) [11] by fluorescence and spectrophotometry. The post-12000 *g* pellet was also used for protein content and enzyme activity catalase A (CAT A) determination.

The results show that yeast grown in presence of IPU in peptone starvation conditions (YED-IPU) exhibit at 72 h a differential growth profile, with cfu, OD, dry weight and level of protein lower than cells grown in YEPD-IPU. The same type of response was detected in terms of antioxidant power estimated by the GSH/GSSG ratio and ability to scavenge free radicals detected by DPPH, as well as the levels of enzyme activities CAT T, CAT A, GR and G6PD which appears much lower in yeast cells grown in YED-IPU medium. On the other hand, cells exposed to IPU in YEPD medium exhibited fermentative activities, ADH and LDH, higher than those detected in cells exposed to phenylurea in the restrictive nitrogen medium, YED-IPU. This set of results suggests that yeast grown in rich medium, YEPD-IPU, remained more fermentative than those grew up in restrictive YED-IPU medium. This interpretation maybe confirmed by higher levels of glutathione and MDA contents, as well as enzyme activities GPx and MDH2 detected in *S. cerevisiae* exposed to YEPD-IPU which started early the respiratory-fermentative transition. So, *S. cerevisiae* grown in the nitrogen starvation conditions may more easily recognize isoproturon as substrate and expand its fermentative phase.

Keywords: peptone; phenylurea; yeast; glutathione; malate dehydrogenase; alcohol dehydrogenase.

References

[1] Lowry O, Rosebrough N, Farr A, Randall R (1951) *J. Biol. Chem*. 193, 265-275.
[2] Hissin H (1976) *Anal. Biochem.* 74, 214-226.
[3] Ohkawa H, Ohishi N, Yagi K (1979) *Anal. Biochem.* 95, 351–358.
[4] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne D (2006) *J. Food Comp. Anal.* 19, 669–675.
[5] Beers R, Sizer I (1952) *J. Biol. Chem*. 195, 133-140.
[6] Goldberg D, Spooner R (1987) *Methods of enzymatic analysis* 3rd ed. Bergmayer, VCH, New York, 258-265.
[7] Flohé L, Gunzler W (1984) *Meth. Enzymol*. 105, 114-121.
[8] Postma E (1989) *Appl. Environ. Microbiol*. 55,468.
[9] Hatanaka A (1975) *Agr. Biol. Chem*. 39, 1677-1678.
[10] Wynn J, Ratledge C (1997) *Microbio.* 143, 253-257.
[11] Romano M, Cerra M. (1969) *Biochim, Biophys. Acta 177*, 421.