

P96 - Analysis of the sensitivity of different fungal species to uracil

Rui Silva¹, Tatiana Q. Aguiar¹, Lucília Domingues¹

¹CEB - Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal. (rsilva@ceb.uminho.pt)

The use of auxotrophies as a tool for genetic modification of microbial strains is widely employed both in fundamental (e.g., molecular characterization of genes based on reverse genetics' approaches) and applied research (e.g., maintenance of plasmids used for the expression of genes of interest). Moreover, the manipulation of auxotrophic strains is a common practice in microbiology laboratories. Among them, one of the most prominent auxotrophies used is that for uracil. In this case, the gene encoding the essential enzyme orotidine-5'-phosphate decarboxylase of the de novo biosynthesis of pyrimidines pathway is deleted (e.g., URA3 gene in *Saccharomyces cerevisiae* and *Ashbya gossypii*, pyrG in *Aspergillus nidulans*) conferring to the mutants the incapacity of growing in medium lacking uracil. Consequently, these auxotrophic strains require the supply of exogenous uracil to compensate their nutritional deficiency. In some studies exogenous uridine supplementation is used instead of uracil. Recently, the presence of high levels of uracil showed to affect the growth of wild and uracil auxotrophic strains of *A. gossypii* and *A. nidulans* [1,2]. However, this sensitivity was more pronounced in the uracil auxotrophic strains than in their respective parent strains. Moreover, the *A. gossypii* Agura3 strain was unable to grow at the same level of the parent strain when the medium was supplemented with uracil alone, being necessary the addition of uridine to overcome this effect [3]. Given the importance and the prominence of these uracil auxotrophic strains, it is of extreme pertinence to elucidate this sensitivity to uracil in *A. gossypii* and analyze if this effect is widespread among different fungal species. It is believed that the cellular levels of uracil must be firmly controlled in order to avoid its incorporation into DNA [2]. Therefore, in this work we systematically characterized the physiological sensitivity of *A. gossypii* and other fungal species (auxotrophic and prototrophic) to uracil and verified that uridine is more adequate for growth supplementation of uracil auxotrophic strains.

REFERENCES: [1] Silva et al., J Biotechnol 2015. 193, 37–40. [2] Sun et al., Sci China Life Sci 2013; 56, 467–75. [3] Aguiar et al., Fungal Genet Biol 2014; 68, 1–8.

ACKNOWLEDGMENTS: to Fundação para a Ciência e a Tecnologia (FCT), Portugal: strategic funding of UID/BIO/04469/2013 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684), Project RECI/BBB-EBI/0179/2012 (FCOMP-01-0124-FEDER-027462) and PhD grant PD/BD/113812/2015 to R Silva.