Poster Session 4: Cell and Metabolic Engineering for New and Improved Protein Production **P4.45**

DELTA MULTICOPY INTEGRATION FOR IMPROVED β-GALACTOSIDASE PRODUCTION IN RECOMBINANT *SACCHAROMYCES CEREVISIAE*

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The β-galactosidase industrial production is hampered by the high costs associated with its production and purification. One way to improve the overall productivity of β-galactosidase fermentation system would be to use continuous high-cell-density systems. Among these, the ones that use flocculent cells are surely attractive due to its simplicity and low cost. We have previously reported the construction of a flocculent *Saccharomyces cerevisiae* strain secreting high levels of *Aspergillus niger* β-galactosidase[1]. Due to the cell flocculation characteristics, the recombinant yeast may be used in a high-cell-density system operating in continuous mode. However, when operating at high dilution rates we have observed some plasmid instability which led to a decrease in the β-galactosidase production[2].

With this work we aim at obtaining stable yeast transformants with at least the same β-galactosidase production level of the previously constructed strain[1] (construction based on an episomal plasmid) but with enhanced stability which would allow to increase the enzyme productivity in the continuous system. For that, the *lac*A gene from *A. niger* (coding to β-galactosidase) was integrated into the genome of the flocculent yeasts *S. cerevisiae* NCYC 869 and *S. cerevisiae* NCYC 869-A3 (ura$^-$) using integrative vectors with a G418 and ura3 marker, respectively. The repeated cromossomal δ sequences of the yeasts were employed as target sites for the integration. The *S. cerevisiae* NCYC 869 integrants were selected by resistance to the aminoglycoside G418 (0.2-1.5 g/l) while for the auxothrophic strain *S. cerevisiae* NCYC869-A3 the selection of integrants was made on minimal medium. Some transforming colonies that presented a deep blue tonality (due to the presence of the X-gal in the plates of selective medium) were randomly selected for growth in non-selective liquid media containing lactose or glucose. Different levels of β-galactosidase expression were observed independently of the selection marker used. For ones that presented more enzyme activity, expression levels of β-galactosidase, cell growth and substrate consumption were found to be similar with the previously constructed strain (with a 2μ-based plasmid). Unexpectedly, the flocculation of the original strains was affected by the integration. The most flocculants were from transformation using the ura3 marker selection system and the second ones were from transformation using 1.5 g/l G418 as selective marker. Nevertheless, all transformants were less flocculent when compare with the original strain.

Transformants genetic characterization by Southern analysis confirmed the multicopy tandem integration pattern. For the analysed transformants, one or two different integration sites were observed. For the most promising transformants, physiological and genetic characterization is being conducted in order to select for a new recombinant strain to be used in a continuous high-cell-density β-galactosidase producing system.

[1] Domingues L et al. Construction of a flocculent *Saccharomyces cerevisiae* strain secreting high levels of *Aspergillus niger* β-galactosidase. *App Microbiol Biotechnol* **58**, 645-50 (2002).
[2] Domingues L et al. *Aspergillus niger* β-galactosidase production in a continuous high yeast cell density reactor. Process Biochem (in press).