OPTIMUM CONDITIONS FOR THE ISOLATION OF *Kluyveromyces marxianus* MUTANTS

Liliana Basabe, Nelson Cabrera, Vladimir Yong, Javier Menéndez, Julio M Delgado and Luis Rodríguez

Bioindustry Division, Center for Genetic Engineering and Biotechnology, P.O. Box 6162, Havana, Cuba. E-mail address: bioind@ingen.cigb.edu.cu

Introduction

Isolation of mutants from yeast has gained importance due to their usefulness as host for gene cloning and expression. Expression systems in non *Saccharomyces* yeasts have been developed (1). One of these yeasts, *K. marxianus* is an industrial attractive yeast. Mutants from this GRAS microorganism have been isolated by non classical procedures (2). In this work, we describe the isolation and characterization of auxotrophic mutants from *K. marxianus* NRRLY 1109 by classical methods. We show that different mutants can be obtained by our procedure and that *K. marxianus his* mutants may be used to develop an expression system in this yeast.

Materials and Methods

K. marxianus strain NRRLY 1109 and *S. cerevisiae* strain SEY 2202 were used. Plasmid pKRHIS (pKR1B (3) with the *S. cerevisiae his3* gene) was used for transformation. Chemical mutagenesis was done essentially as described by Haas, *et al.* (4). For UV light mutagenesis cells were grown on YPD medium up to 10^8 cells/mL, washed in NaCl (0.9 %). After growing on YPD for 48 h, cells were washed with distilled water for nystatin enrichment or in 50 mM sterile phosphate solution, pH 5.5 for snail enzyme procedure. 10^7 cells were incubated in YND at 30 °C before adding nystatin or snail enzyme, and incubated at 30 °C for 30 min or 2 h respectively. Phenotypically similar mutants were crossed on YPD and replicated onto YND containing plates. Some mutants were transformed and transformants were checked by plating into a G418 containing plate (700 µg/mL) or by southern blot analysis.

Results and Discussion

Optimal doses of mutagens were determined. Cells were irradiated 2 min or incubated with NTG, 50 µg/mL. Either by UV or NTG mutagenesis we obtained mutants at high frequencies (>62 %). Nystatin or snail enzyme optimal concentrations were established (5 U/mL and 2 % respectively). Enrichment performed either with the antibiotic or the enzyme incubating cells on YND for 4 h before adding them, showed no differences on phenotype variety obtained (only *his* mutants with a low reversion frequency: 10^{-7}-10^{-8}). A likely explanation could be not optimal enrichment conditions have been used, probably due to the incubation time on YND before adding the antibiotic or the enzyme. In this case these times had great influence on the obtained phenotypes (Table 1). The best results were achieved for a propagation time of 2 h. It has been reported for fungi the significance of propagation step, before adding the lytic enzyme or the antibiotic, for the enrichment efficiency, specially for these methods based on selective killing (5). These results showed this step is essential for enrichment efficiency in yeast. This procedure under appropriate conditions proved to be efficient for isolating auxotrophic mutants from *K. marxianus*.

Table 1. *Auxotrophic mutants isolated by UV mutagenesis and nystatin enrichment by trying different incubation times before adding nystatin and percentage of each auxotrophy obtained.*

Incubation time (h)	Auxotrophy	Percent
	Histidine	92
	Methionine	3.8
2	Isoleucine	1.9
	Asparagine	0.6
	Tryptophan	0.6
	Lysine	0.3
4	Histidine	100
6	Histidine	100
8	Histidine	100

Complementation tests showed all predominant *his* mutants belong to the same complementation group. Transformation of these food grade mutants with pKRHIS showed *S. cerevisiae his3* gene is functional in *K. marxianus*. Transformants showed growth onto a G418 containing plate unlike the wild type strain. Southern blot revealed the presence of the replicative pKRHIS plasmid. Non revertant were detected. The data show that mutants used for transformation are stable *his3* mutants, which might be used for developing an efficient host vector system in *K. marxianus* based on a new auxotrophic marker for this strain.

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3. Sreekrishna K, *et al.* Gene 1984; 28: 73-81.

4. Haas LOC, *et al.* J Bacteriol 1990; 172 (8):4571-4577.

5. Bos CJ, *et al.* Curr Genet 1992; 21: 117-120.