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UV-induced curing of the double-stranded RNA virus of the yeast *Yarrowia lipolytica*

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Summary. Curing of the viruslike particles harbored by a strain of the yeast *Yarrowia lipolytica* was achieved by UV irradiation. The cured strain was found to be able to maintain the viruslike particles after their re-introduction by crossing or by cytoplasmic fusion. The involvement of a UV-induced mutation of a yeast maintenance gene seems therefore unlikely.

Key words: *Yarrowia lipolytica* – Viruslike particles – Curing – UV light

Introduction

Numerous species of yeasts and fungi harbor viral particles, but generally their study is limited by their lack of infectivity and by the absence of a host phenotype related to their presence. In this respect, the killer system of *Saccharomyces cerevisiae* appears as an exception: because of the easy-to-observe killing ability of yeast strains possessing some combinations of VLPs, it has become one of the best systems to study host-virus interactions (Tipper and Bostian 1984). Several conditions have been described which result in the loss of at least one of the two viral dsRNAs present in the cells of killer strains, and subsequently in sensitivity of the strains so "cured" (Fink and Styles 1972; Mitchell et al. 1976; Wickner 1974). The cured strains are still able to maintain viral dsRNAs (Wickner 1974; Bevan and Mitchell 1979). Loss of the killer character can also result from a mutation in any of numerous host nuclear genes (MAK genes, for "maintenance of killer"), giving rise to a strain unable to maintain at least one of the two viral dsRNAs (Tipper and Bostian 1984).

Recently, the presence of dsRNA-containing VLPs was reported in the yeast *Yarrowia lipolytica* (Groves et al. 1983). They do not seem to affect their host or to confer any killing ability (Tréton et al. 1985). Nevertheless it was possible to monitor the efficiency of curing experiments, by detecting viral dsRNA after extraction of total nucleic acids. Results of such assays are exposed here.

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Abbreviations. UV ultraviolet; *dsRNA* double-stranded RNA; *VLPs* viruslike particles, *A* and *B* alleles of the mating type locus; *TCA* trichloracetic acid

Materials and methods

Yarrowia lipolytica strains. CBS 6124-2, a wild-type collection strain of B mating type, was obtained from the Central Bureau vor Schimmelculture, Delft, the Netherlands. MK2-01 (B lys$^-$ met$^-$) carries an oligomycin resistance marker showing cytoplasmic inheritance. It was kindly given by Dr. Matsuoka M., Osaka, Japan. 8051-9 (A lys$^-$) and 14701-10 (A his$^-$) are from our laboratory. All other strains are described in the text.

Growth media. Rich media were YEPD (1% yeast extract, 1% bactopeptone, 2% dextrose) or YEA (0.5% yeast extract, 2% dextrose). Minimal medium was YNB (0.67% Difco Yeast Nitrogen Base, 1% dextrose). Upon requirement, it was adjusted to pH 5.0 with 50 mM citrate-phosphate buffer. For supplementation of auxotrophies, 100 mg of required aminoacid(s) were added per liter. When necessary, media were gelified with 1.4% agar.

Growth monitoring. Turbidity of cultures was estimated with a photoelectric Klett-Summerson colorimeter.

Nystatin selection. As in Beckerich et al. (1984).

Genetic analysis. Crosses and progeny analysis were performed as in Gaillardin et al. (1973).

Table 1. Results of curing attempts

Treatment	Conditions or dosis	Survival rate	VLP-free colonies/tested colonies
Growth on cycloheximide	0.1 to 2 mg/ml	100%[a]	0/60
Growth on 5-fluorouracil	1 to 40 mg/ml	26%[a]	0/60
Growth at elevated temperature	30 to 34 °C	60%[a]	0/144
Exposition at 40 °C before growth at 30 °C	20 s to 3 min	0.31%[a]	0/60
UV irradiation (2,000 ergs/cm^2)	5 s	5%	0/50
	15 s	0.1%	1/50
	30 s	0.001%	0/50

[a] lowest survival rate

Table 2. Genetic analysis

A. Crosses	Strains	VLP-free colonies/ tested diploids	VLP-free colonies/ tested segregants
	CBS 6124-2C/1 x 8051-9	0/60	0/50
	CBS 6124-2C/1 x 14701-10	0/60	0/50

B. Cytoplasmic fusion	Strains	Type of colony	Number	Ploidy[a]	VLP-free colonies/ tested colonies
	CBS 6124-2C/1 + 53	prototroph	4	2.1	0/4
		arg$^-$ auxotroph	112	1.0	0/21

[a] Estimated by DNA content measurement (Burton 1956), and compared to mean value of 3 haploid strains, CBS 6124-2, CBS 6124-2C and 53

Protoplast obtention and fusion. As in Beckerich et al. (1984), with sorbitol as an osmotic stabilizer. Protoplasts were regenerated on the surface of solid medium, without top agar.
For obtention of cytoplasmic hybrids, regeneration of fusion products was achieved on minimal medium supplemented with arginine and containing oligomycin (50 mg/l) and 1% glycerol as a carbon source.

Measurement of cell DNA content. Cells were grown to early stationary phase in YEA medium. After extraction with hot TCA, DNA concentration was estimated by the diphenylamine method of Burton (1956), using deoxyadenosine as a standard.

All *experiments concerning viral and cellular RNAs* were performed as in Tréton et al. (1985).

Results and discussion

We were interested in getting a strain devoid of VLPs but still able to maintain them. Although it was possible to screen virus-free strains of *Y. lipolytica* for the presence of efficient maintenance alleles by performing cytoplasmic fusions with a strain harboring VLPs, we chose to test curing methods on a VLP-containing strain, CBS 6124-2. To monitor their efficiency, extraction of total RNAs was performed on treated colonies. After agarose gel electrophoresis, viral RNA band was routinely detected by ethidium bromide staining, then by hybridization with ^{32}P-labeled viral RNA from CBS 6124-2 when necessary.

As can be seen from Table 1, methods resulting in high frequency curing of *S. cerevisiae* killer determinant(s) were inefficient on *Y. lipolytica* VLPs, or not efficient enough to ensure curing with a frequency allowing its detection on the small colony number we were able to test. On the contrary, elimination of VLPs from CBS 6124-2 was obtained after UV irradiation. A rough correlation was observed between survival rate and viral RNA amount in surviving colonies, both decreasing when irradiation time was increased. Nevertheless, the only colony which was found totally devoid of VLPs (at the sensitivity level of our detection methods) did not arise from the most irradiated cells. We called it CBS 6124-2C.

Growth rates of CBS 6124-2 and CBS 6124-2C were measured at 28 °C. CBS 6124-2C grew slightly slower than CBS 6124-2 (doubling times in YEA and YNB: respectively 2 h 25 and 5 h instead of 2 h 10 and 4 h 15), but cell densities at the plateau were identical. No differences in thermosensitivity were observed between both strains.