

genome (M dsRNA) of a mycovirus. These RNA plasmids are found only in killer strains, and these contain a second genome of the mycovirus (L dsRNA). Both types of dsRNA exist in cytoplasmic virus-like particles. The L dsRNA encodes for the production of the capsid protein for both virus particles. Detailed analysis of L dsRNA isolated from *Saccharomyces* strains showed that three distinct, linear species are found (L_A , L_B , L_C) which are all similar in size (about 4.7 kb). The presence of both L and M dsRNA is necessary for the expression of killer character, whereby L_A dsRNA is necessary for the maintenance of M dsRNA. None of the brewing strains exhibits killer character. Therefore, there is a considerable potential for exploiting killer systems, e.g. in fermentation processes, and a demand for protecting yeasts of industrial interest against contamination with killer strains. The most elegant way is the transfer of the killer character into commercial yeasts by plasmid injection because this has decisive advantages over transmission of plasmids by yeast protoplast fusion [4].

Attempts to inject dsRNA by chemical means have proved unsuccessful. Therefore, incorporation of M dsRNA isolated from a killer strain was accomplished using the electroinjection technique introduced by Zimmermann et al. [5,6] and modified by Stopper et al. [7,8].

3. MATERIALS AND METHODS

3.1. Yeast protoplasts

Yeast protoplasts were prepared from the *Saccharomyces cerevisiae* laboratory mutant strains AH 215 (leu^- , his^-) and from an industrial haploid *S. cerevisiae* strain AS-4/H2 (ρ^-) as well as from a killer-negative variant of the super-killer *S. cerevisiae* mutant strain T 158 C (his^-) using standard protocols [9,10]. These strains did not contain M_1 dsRNA (Fig. 1) and therefore exhibited no killer activity. The protoplasts were suspended at a density of 10^9 cells/ml in a solution containing 1.2 M sorbitol, 30 mM KCl, 1 mM $CaCl_2$, 0.3 mM KH_2PO_4 , 0.85 mM K_2HPO_4 and 10 μ g/ml isolated M_1 and L_1 dsRNA.

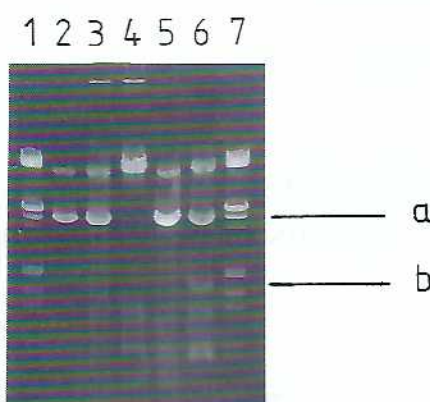


Fig. 1. Agarose-gel electrophoresis of nucleic acid extracts from yeasts. Tracks 1 and 7, standard DNA (21.7-, 5.15-, 5.0-, 4.27-, 3.48-, 1.98-, 1.9-, 1.59-, 1.37-, 0.94- and 0.83 kb); track 2, super-sensitive strain S 6-1; track 3, sensitive (laboratory) strain AH 215; track 4, sensitive (industrial) strain AS 4/H2; track 5, killer-negative variant of the K_1 killer strain T 158 C; and track 6, K_1 super-killer strain T 158 C. a, L_{1A} dsRNA; b, M_1 dsRNA. Conditions: 1% agarose, Tris-acetic acid EDTA-buffer (TAE-buffer: 40 mM Tris, 2 mM EDTA pH 8.3 adjusted by acetic acid), 80–100 V, 50–60 mA, ethidiumbromide staining.

3.2. dsRNA isolation

The dsRNA for K_1 toxin was isolated from the killer strain of *S. cerevisiae* T 158 C (his^-) according to the procedure described by Fried and Fink [11] with the following modifications. In order to remove proteins quantitatively from the nucleic acids the crude cell extract was incubated and gently shaken for 15 min in 50 mM Tris- H_2SO_4 (pH 9.3) containing 2.5% 2-mercaptoethanol and for 1 h in solution I containing 0.1 M NaCl, 10 mM Tris-HCl (pH 7.5), 10 mM Na_2EDTA , 0.2% sodium dodecylsulphate and an equal volume of bi-distilled phenol. After 20 min centrifugation at $5000 \times g$, nucleic acids were recovered from the aqueous phase by precipitation with about 2.5 volumes of 95% ethanol and stored at 4°C for 24 h. After 30 min centrifugation at $16000 \times g$ the pellet was dissolved in solution I. After removal of the phenol phase, the aqueous phase was mixed with an equal volume of chloroform (purified by pre-treatment with TE buffer) and shaken for 10 min. 0.1 volume of 2.5 M sodium acetate (pH 6) was added to the aqueous phase and the nucleic acids precipitated by adding 2.5 volumes of 95%

ethanol. centrifuged at $16000 \times g$ for 30 min. The supernatant was dissolved in 0.15 M NaCl. At least 8 h after centrifugation the supernatant was removed. The remaining supernatant was subjected to a second centrifugation in 95% ethanol. The supernatant was dissolved in 0.15 M NaCl. 1 ml slices of M_1 dsRNA were subjected to electroinjection (100 V) and combined with L_{1A} dsRNA and ethanol. The mixture was stored at 4°C until use. The supernatant was removed.

From Fig. 1 it is evident that the M_1 dsRNA (which is present in the K_1 and L_{1A} dsRNA) is not present in the laboratory and industrial strains.

3.3. Electroinjection

For electroinjection the cells were prepared as described above. The cells were exposed to a square wave electric field of 18.2 kV/cm for 100 ns at 20°C. The cells were then washed with distilled water (Biojet M) and the cells were distributed into petri dishes. The cells were exposed to 100 pulses with a duration of 100 ns. These conditions were found to be suitable for electroinjection. After pulsed cells were washed with distilled water. After pulsed cells were mixed with distilled water in petri dishes.

3.4. Regeneration

The cells were grown on agar medium without a carbon source. The cells were grown in 10 mM histidine. The cells were grown on 2% agar medium.