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 maintainance 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 (rho^-) 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 μg/ml isolated M_1 and L_1 dsRNA.

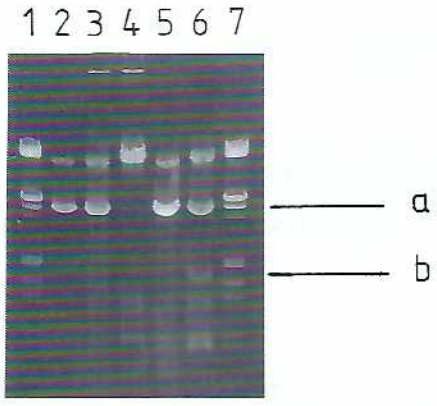

Fig. 1. Agarose-gel electrophoresis of nucleic acid extracts from yeasts. Tracks 1 and 7, standard DNA (21.7-, 5.15-, 5.0-, 4.27-, 3.48-, 1.98-, 1.9-, 1.59-, 1.37-, 0.94- and 0.83 kb); track 2, super-sensitive strain S 6-1; track 3, sensitive (laboratory) strain AH 215; track 4, sensitive (industrial) 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 aqeuous phase and the nucleic acids precipitated by adding 2.5 volumes of 95%