actidion (0.89 μM). This treatment resulted in the removal of M dsRNA from the cells (Fig. 1) and, therefore, in the loss of the killer activity.

This model transformation system was used because it allowed conclusions about the frequency of electrically-mediated dsRNA transfer. The yield of transformed clones should not be limited by hindered expression of transmitted dsRNA in the host protoplasts.

The protoplasts were subjected to three consecutive field pulses of 18.2 kV/cm strength and 40 μs duration. After pulsing of the protoplasts of the killer-negative variant of *S. cerevisiae* T 158 C at either 4°C or at 20°C about 10^3 to 10^4 cells could be regenerated. Screening for killer activity by using the selection medium plus cells of the super-sensitive strain *S. cerevisiae* S 6-1 revealed that the majority of these protoplasts were transformed (Fig. 2a). On average 14% of the clones exhibited weak killer activity and 82% super-killer activity when electroinjection was conducted at 4°C. Since the killer activity apparently depends on the number of plasmid copies (about 10 to 12) [1,2], the occurrence of transformed cells with weak killer activity indicates either that these cells had received only one or a few plasmids due to electropermeabilisation, that replication of electrically-transmitted plasmids was disturbed or that the structure of the dsRNA was modified during uptake.

Similar yields was obtained when the dsRNA was added immediately after pulsing, indicating that the field pulses had no adverse side effects on the plasmids.

Variations of the field strength and the number as well as of the duration of the field pulses resulted in a significant decrease of transformed clones. Pulsing at room temperature also generally resulted in a reduction of transformed clones (about 2% showing weak killer activity and about 25% exhibiting super-killer activity). This temperature-dependence of the yield is consistent with previous results and can be explained by the temperature-dependence of the resealing process of the field induced perturbations within the membrane [12].

In the second set of experiments we investigated the electric-field mediated injection and expression of the M_1 dsRNA in the sensitive laboratory and industrial *Saccharomyces* strains mentioned above under the same conditions.

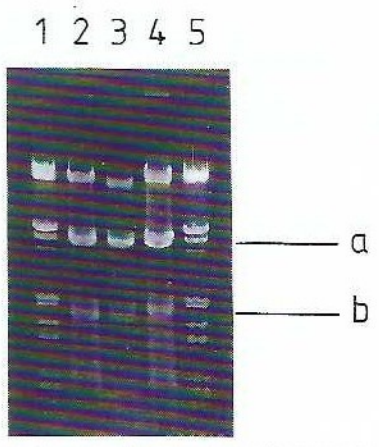

Fig. 3. Agarose-gel electrophoresis of dsRNA of yeast cells transformed with M1 dsRNA isolated from the super-killer strain T 158 C. Tracks 1 and 5, standard DNA; track 2, transformed, killer-negative variant of T 158 C; track 3, transformed AH 215; and track 4, transformed strain AS 4/H2.

As shown in Figs. 2b and c transformants were also obtained from both strains (including super-killer clones of the industrial strain). However, the yield of transformed clones was much less than in the model transformation experiments, particularly at 4°C. This result is expected if the expression of the electroinjected dsRNA was diminished. This conclusion was supported by preliminary experiments using dsRNA preparations from killer types K_2 and K_3. It was found that some of these dsRNA preparations did not lead to any transformants.

Gel electrophoresis of the dsRNA of some of the obtained transformed clones shown in Figs. 2a–c supported the conclusion that the transformed clones exhibited killer activity (Fig. 3).

The transformed clones of the model transformation system and of the laboratory and industrial strains were stable. After about 4 weeks they showed no significant change in killer character.

The results reported here demonstrate for the first time that the killer character can be transferred directly into laboratory and industrial yeast strains when the electroinjection method is used.

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