

Electrophoretic karyotype of budding yeasts with intact cell wall

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Intact yeast cells subjected to a punctual electric field (in an electroporator) can be transformed with exogenous DNA (1), indicating that DNA molecules can move into the cell through the cell wall. In pulsed-field electrophoresis, we have also observed that removal of the cell wall is an unnecessary step for the large chromosomal DNA molecules (ranging from 2000 to 240 Kb), as well as for mitochondiial DNA ones (~70 Kb), to be able to move out of the cell.

The most widely used method of sample preparation for pulsed-field gel electrophoresis of yeasts involves cell wall digestion (2). The yeast cells, washed once with EDTA 50 mM, are entrapped in 0.5% agarose plugs at a final concentration of $2-3\times10^9$ cells/ml, with 0.1 mg/ml Zymolyase, Na_2PO_4 60 mM, EDTA Na_2 10 mM, D-Sorbitol 0.6 M, Dithiothreitol 0.5 mM, Citric acid 20 mM pH 6. The entrapped cells are then incubated during 6 hours at 37°C in Na_2PO_4 60 mM, EDTA Na_2 10 mM, Citric acid 20 mM pH 6, to allow cell wall digestion and cell lysis. Bellis *et al.* (3) have shown that this step can be replaced by 6 hours incubation at 37°C with 0.5 M β-mercaptoethanol, NaCl 0.5 M, EDTA Na_2, Tris−HCl 0.125 M pH 7.5 (conditions in which spheroplasts can be obtained bypassing the expensive Zymolyase). Plugs with lysed cells are incubated for 12 hours at 50°C with 1% Lauroyl Sodium Sulphate and 1 mg/ml of Proteinase K in EDTA Na_2 450 mM, Tris−HCl 10 mM pH 8. Before running the electrophoresis, plugs are washed three times for 30 minutes in TE at 50°C, and six times more in 0.5×TBE (electrophoresis buffer) at room temperature.

We have observed that the cell wall digestion and cell lysis step can be omitted. Figure 1 shows the result obtained when the 6 hours Zymolyase + Dithiothreitol treatment is applied, compared to that obtained by eliminating this step. Cells from plugs treated with Zymolyase + Dithiothreitol were lysed, while those from plugs where this step was eliminated maintained their shape, with the cell wall intact (Figure 2). We conclude that molecules of DNA can freely move into and out of the cell through the cell wall when subjected to electrical fields, and therefore, in the case of the pulsed-field electrophoresis, the time-consuming step of the Zymolyase + Dithiothreitol or β-mercaptoethanol treatment can be omitted, with no effect on the electrophoretical karyotype of budding yeast.

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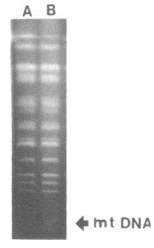

Figure 1. Electrophorectic karyotype of the budding yeast *Saccharomyces cerevisiae* using a CHEFF apparatus. A) The yeast cell wall was digested with Zymolyase + Dithiothreitol, following the current protocol of sample preparation. B) The Zymolyase + Dithiothreitol treatment was omitted. The mitochondrial DNA is indicated.

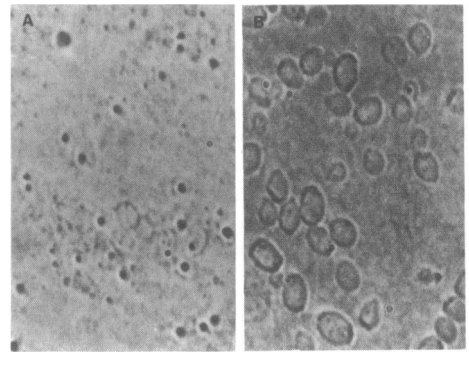

Figure 2. Micrographs of the entrapped cells yielding the electrophorectic karyotype A and B of Figure 1. A) Lysed cells obtained after the Zymolyase + Dithiothreitol treatment. B) Cells with intact cell wall.

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