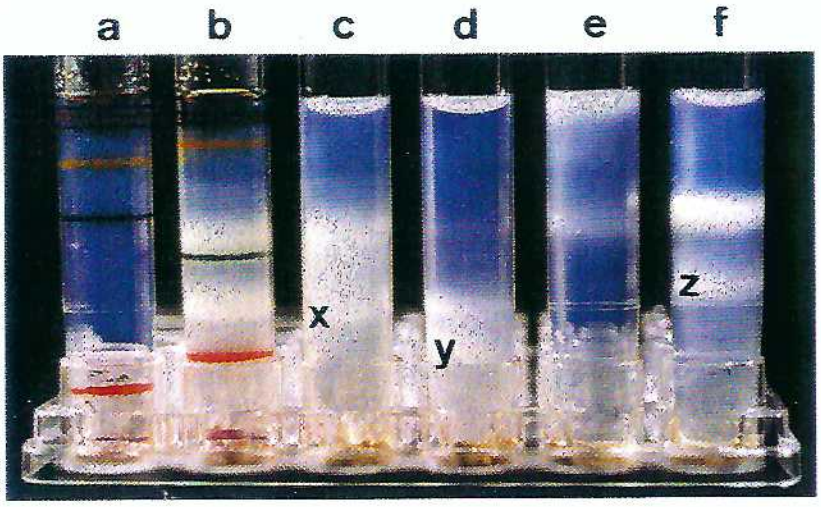

Fig. 1. Separation of enucleated protoplasts/spheroplasts from discontinuous density gradient of Percoll: a – density marker beads, from bottom: violet (d = 1.142 g/ml), red (1.121 g/ml), green (1.098 g/ml), orange (1.087 g/ml), blue (1.075 g/ml); b – separated spheroplasts of *S. carlsbergensis* 34, *rho*$^+$ strain, synchronized by nocozadole and treated with CB (enucleation medium II); cytoplasts were taken from a band of nucleated cells (d = 1.11 g/ml) between the red and green density marker beads; c – protoplasts of *S. carlsbergensis 34 rho*$^+$ strain, treated by CB in enucleation medium I, w/o density marker beads; cytoplasts were taken from a band marked "x"; d – separated spheroplasts of *S. cerevisiae* H_3, *rho*$^+$ strain; cytoplasts were taken from the band marked "y" (d = 1.12 g/ml); e – the band of enucleated cells (big buds w/o nucleus) from *S. carlsbergensis* 34, ***rho***$^-$ strain, w/o density marker beads; f – separated cells from *S. carlsbergensis* 34, ***rho***$^-$,w/o density marker beads; cytoplasts were taken from the band of nucleated cells, marked "z".

Fig. 2. Fluorescence micrographs of DAPI-stained spheroplasts (from non-synchronized culture) before enucleation: a – *Saccharomyces uvarum* var. *carlsbergensis* 34 *rho*$^+$; b – *Saccharomyces uvarum* var. *carlsbergensis* 34 ***rho***$^-$; c – *Saccharomyces cerevisiae* H_3 *rho*$^+$. The large bright spots are cell nuclei, whereas the small spots visible only in a) and c) are mitochondria, b) was exposed under the same conditions as a) and c); the uniform intracellular background in b) is typical of other ***rho***$^-$ strains (not shown). In all micrographs, the bar represents 8 μm.

Fig. 3. Fluorescence micrographs of DAPI-stained protoplasts (top row) and spheroplasts (bottom row) after enucleation using enucleation medium I (w/o synchronization by nocodazole, but including discontinuous density gradient centrifugation): a – *Saccharomyces uvarum* var. *carlsbergensis* 34 *rho*$^+$; b – *Saccharomyces uvarum* var. *carlsbergensis* 34 ***rho***$^-$; c – *Saccharomyces cerevisiae* H_3 *rho*$^+$. The large bright spots visible in some cells are nuclei, but the small spots in *rho*$^+$ strains are mitochondria. In all micrographs, the bar represents 8 μm.

Fig. 4. Fluorescence micrographs of DAPI-stained spheroplasts after enucleation using enucleation medium II (including discontinuous density gradient centrifugation): a – *Saccharomyces uvarum* var. *carlsbergensis* 34 *rho*$^+$; b – *Saccharomyces uvarum* var. *carlsbergensis* 34 ***rho***$^-$; c – *Saccharomyces cerevisiae* H_3 *rho*$^+$. Large bright spots visible in some cells are nuclei, but the small spots in *rho*$^+$ strains are mitochondria. In all micrographs, the bar represents 8 μm.

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