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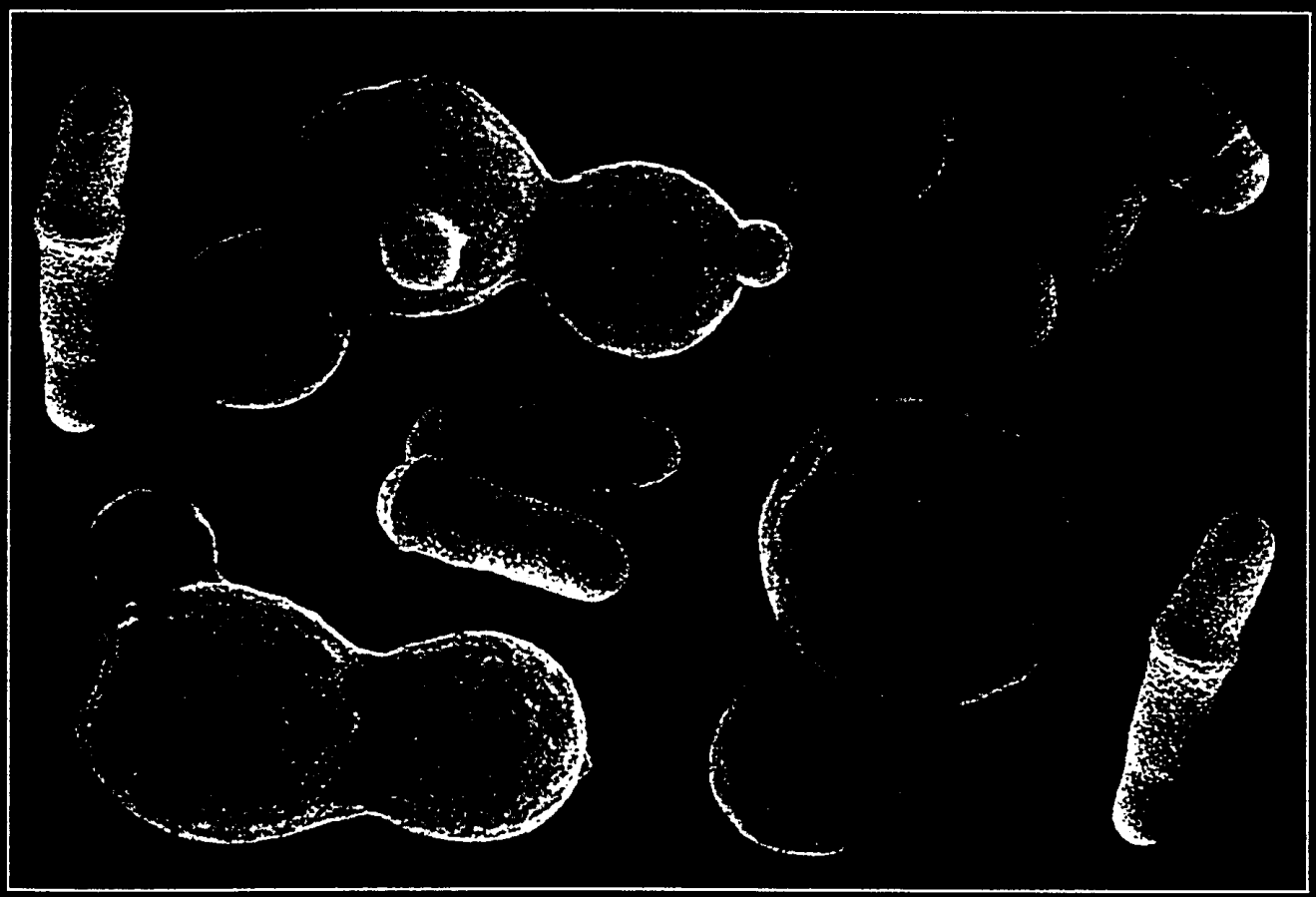

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GENETIC TRANSFORMATION OF INTACT INDUSTRIAL FLOCCULATING YEAST CELLS (*SACCHAROMYCES CEREVISIAE*) BY USING LITHIUM ACETATE AND YAC4 PLASMID

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Transformation was first performed in bacteria by Griffith in 1928, and the transforming molecule was discovered to be DNA by Avery, McLeod and McCarty in 1944. The ability of *Saccharomyces cerevisiae* spheroplast to take up exogenous plasmid DNA was accomplished by Hinnen and col. and Beggs in 1978. These results are milestones in molecular biology.

Nowadays several methods to transform DNA into yeast cells are available such as transformation of spheroplasts made with lytic enzymes, transformation of intact cells made competent by lithium acetate or by electroporation.

According to our knowledge there are no published methods for preparing YAC clones with simpler transformation protocols as the lithium acetate method. We report here, for the first time, the use of YAC4 plasmid - a yeast artificial chromosome shuttle vector containing the yeast *URA3* gene as selectable marker - to transform intact *Saccharomyces cerevisiae* cells using lithium acetate and single-stranded carrier DNA. Furthermore, as the fermentation of high cell densities provide the possibility to produce proteins in sufficient amounts for the establishment of industrial isolation and downstream processing procedures we used in this study a flocculent *Saccharomyces cerevisiae* strain NCYC-869 (*Matα Flo1*) with *ura3* constructed by ultraviolet mutagenesis and tested for their resistance towards 5-fluoro-orotic acid.

In this work, the transformation efficiency was higher than 10^3 transformants per microgram of plasmid DNA which is comparable to other described yeast transformation systems.

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