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A Study of Copulation, Sporulation and Meiotic Segregation in *Candida lipolytica*

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Summary. We have attempted to optimize conjugation and sporulation in *Candida lipolytica*, by studying the conditions of culture and growth.

Copulation between compatible strains is a rare event, particularly in the case of auxotrophic mutants. However, diploids can be selected for on minimal medium provided parents are suitable auxotrophs. These diploids can multiply vegetatively for many generations. They can also be induced to sporulate at a very high frequency.

Free ascospores were isolated by means of paraffin oil and segregations of markers could be studied. At first quite irregular, these segregations improved following a number of brother-sister matings. At the same time, the mean number of spores per ascus as well as spore germinability were considerably increased.

The cell cycle of the yeast *Candida lipolytica* has been elucidated recently, as a result of investigations by Wickerham *et al.* (1969, 1970). This species is usually found in nature as haploid strains belonging to one or the other of two mating types, designated here by A and B. Rarely sporulating diploids are also found.

It could be shown that, on appropriate media, copulation occurs between strains of compatible mating types, followed by the formation of asci containing 1 to 4 spores. The way seemed therefore to be open for a genetical analysis of this yeast.

The main incentive to conduct such investigation on yeasts of the genus *Candida* is their industrial potentialities as a source of single-cell proteins and of various metabolites. However, numerous problems remain to be solved such as increasing the frequency of asci in crosses, the number of spores per ascus and the germinating capacities of the spores.

Independently of the work reported here, several investigators have studied these problems as well. Herman (1971) has described conditions allowing the formation of zygotes between compatible strains. Bassel *et al.* (1971) reported their first results on the possibilities of complementation and recombination in *Candida lipolytica*, confirming the existence of a diploid stage, the meiotic origin of sporal clones and the control of the mating types by two alleles of a single locus.

We have investigated the possibilities of better controlling the various stages of the life cycle of this yeast, especially conjugation, complementation and sporulation. This led us to study means of improving the wild strains genetically, so as to produce derivatives whose subsequent behaviour would facilitate their use in fundamental or applied research.

Material and Methods

Strains. We used initially a wild strain W, isolated from soil. It was shown to be of mating type A.

The first compatible strains placed at our disposal were sent to us by Wickerham (US Department of Agriculture, Peoria, Illinois): 423-3 of type A and 423-12 of type B. This last strain will be here after designated Z.

Several auxotrophic mutants were induced in strains W and Z. Those used in this study are listed in Table 1.

After cross-breeding, we isolated a number of doubly heterozygous diploid strains, which are listed in Table 2.

Media and Culture Conditions. Most of the media used in this study have already been described. The complete (YE) and minimal (MM) media are those of Leupold (1955) slightly modified. YE medium contains 2% glucose, 0.5% Difco Bacto Yeast Extract, 2% Difco Bacto Agar; MM medium: 1% glucose, 100 ml A2 salts, 2% Difco Bacto Agar and thiamine at a final concentration of 10 µg/l. Minimal medium may be supplemented with amino acids or purine pyrimidine bases (all at 10^{-3} M), depending on the nutritional requirement of the auxotrophic mutant used.

In order to improve sporulation, the strains to be crossed are precultivated for 24 h on a pre-sporulation medium (PSM), of the following composition: 2% glucose, 0.5% $(NH_4)_2SO_4$, 0.5% Difco Yeast Extract, 0.2% KH_2PO_4, eventually solidified with 2% Difco Agar.

Several sporulation media have been used: MC medium containing CH_3COOK 9.81 g, glucose 1 g, Difco Yeast Extract 2.5 g, NaCl 1.18 g, $MgSO_4 \cdot 7\,H_2O$ 0.71 g, Difco Agar 20 g for 1 l water (Mc Clary *et al.*, 1959); F medium: 0.4% CH_3COONa, 2% Difco Agar (Fowell, 1952); RG medium: 0.02% Difco Yeast Extract, 0.02% Difco Bacto Peptone, 0.1% glucose and 2% Difco Agar (Herman, 1971); YM medium: 0.3% Difco Yeast Extract, 0.3% Malt (Maltea Moser, 45 — Pithiviers), 0.5% Bacto Peptone Difco and 2% Difco Agar (Wickerham, 1951). Medium V8 is adapted from Wickerham (1951): to 1 l of V8 juice (Campbell's Soup, S.A., Felegara, Parma, Italy) at pH 6.8 with KOH are added 200 g of yeast cake. After 10 min in a boiling water bath, the mixture is filtered, diluted with 1 l of tap water and eventually solidified with 2% Difco Agar. A modified version of this medium is V8C medium, in which the yeast cake is replaced by 20 g/l of dried cells (exponential phase) of W strain.

Media are sterilized for 20 min at 120° C. Amino acids as well as purine-pyrimidine bases are filter-sterilized and added later (Millipore filter 0.8 µm diameter).

Cultures on solid medium were usually incubated at 25° C and those in liquid medium at 27—28° C in a giratory shaker (New Brunswick Aquatherm). To separate cell clumps, before dilution or counting in hemacytometer, cell suspensions were sonicated at 0° C (3 min at max. intensity with Mullard's desintegrator). Controls showed that such a treatment did not cause any appreciable mortality.

Induction and Selection of Mutants. Out of an agar slant (MM) inoculated the day before, a suspension was prepared with approximately $5 \cdot 10^5$ cells/ml. 5 ml