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Cytological and Genetic Studies of the Life Cycle of *Saccharomycopsis lipolytica*

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Summary. In the alkane yeast *Saccharomycopsis lipolytica* (formerly: *Candida lipolytica*) the variability in the ascospore number is caused by the absence of a correlation between the meiotic divisions and spore wall formation. In four spored yeasts, after meiosis II, a spore wall is formed around each of the four nuclei produced by meiosis II. However, in the most frequently occurring two spored asci of *S. lipolytica*, the two nuclei are already enveloped by the spore wall after meiosis I due to a delay of meiosis II. This division takes place within the spore during the maturation of the ascus. In this case germination of the binucleate ascospore is not preceded by a mitosis. It follows that the cells of the new haploid clones are mononucleate. In the three spored asci, which occur rarely, only one nucleus is surrounded by a spore wall after meiosis I; the other nucleus undergoes meosis II before the onset of spore wall formation. The result is one binucleate and two mononucleate spores. In the one spored asci the two meiotic divisions occur within the young ascospore, i.e. spore wall formation starts immediately after development of the ascus. These cytological observations were substantiated by genetic data, which in addition confirmed the prediction that binucleate spores may be heterokaryotic. This occurs when there is a postreduction of at least one of the genes by which the parents of the cross differ. This also explains the high frequency of prototrophs in the progeny of non-allelic auxotrophs since random spore isolates are made without distinguishing between mono- and binucleate spores. The possibility of analysing offspring of binucleate spores by tetrad analysis is discussed. These findings enable us to understand the life cycle of *S. lipolytica* in detail and we are now in a position to start concerted breeding for strain improvement especially with respect to single cell protein production.

Introduction

During the last few years various species of the form-genus *Candida* have become of interest in both basic and applied research. This is due to the fact that some *Candida* species are a source of "single cell protein" and can utilise alkanes or methanol as a sole carbon source for growth (for lit. see Yamada et al., 1968; Cooney and Levine, 1971).

The alkane yeast *Candida lipolytica* has been of particular interest. Wickerham et al. (1969, 1970) showed that this fungus could reproduce sexually and that compatibility between strains may be determined by physiological dioecism (for classification of breeding systems see Esser, 1971). That *C. lipolytica* was classified until recently in the Deuteromycetes stems from the fact that most strains isolated from nature are representative of only one of the mating types and can only be crossed under special nutritive conditions (Bassel et al., 1971; Gaillardin et al., 1973). The elucidation of the sexual cycle has led to the reclassification of *Candida lipolytica* as *Saccharomycopsis lipolytica* Wickerham et al., Yarrow nov. comb. (Yarrow, 1972).

The application of genetic analysis to concerted breeding of industrial strains of *S. lipolytica* has been handicapped by two problems (see Gaillardin et al., 1973):

1. The number of spores in each ascus is very variable. Depending on the strain, instead of the expected 4 spores, up to 90% of the asci contain only two spores and a few asci contain 3 or even just 1 spore.

2. In most crosses between non-allelic auxotrophs there are gross deviations from the expected 1:1:1:1 ratio and this is in favour of the prototrophic class.

It was not known whether these irregularities in spore number were due to the development of multinucleate spores as occurs

in some other fungi, or due to the lethality of some nuclei after meiosis as described for many other lower organisms. It was, therefore difficult to interpret segregation patterns in the ascospore progeny.

In order to understand these unusual phenomena we have studied ascospore formation in detail by cytological and by genetical methods.

Material and Methods

Strains. For all experiments we used the two auxotrophic haploid mutants *+his* (serial number I/14) and *−lys* (serial number H-5029/7) of *Saccharomycopsis lipolytica*. By analogy with most other fungi we represent the two alleles of the mating type gene by the symbols + and −. This avoids the capital letters *A* and *B* used by other authors (e.g. Gaillardin et al., 1973) which unintentionally suggests dominance relationships or even non-allelism.

Origin of Strains. We started out with two wild strains of unknown mating type (H-5027, H-5029) isolated from soil by Dr. Präve (Frankfurt) and the auxotrophic mutant kindly given to us by Prof. Heslot (Paris) under the serial number 8051/13 *his A* (according to our classification this mutant has the + mating type).

+his was derived from a cross between the "Heslot-mutant" and the variant − *arg* obtained from H-5027.

−lys is a mutant obtained from wild strain H-5029.

Auxotrophic strains were obtained after UV-treatment of haploid cells (254 nm, 2,400 erg/mm^2).

Media, Culture Conditions and Crossing Methods were carried out according to the methods described by Gaillardin et al. (1973). Asci and ascospores were isolated using a Leitz micromanipulator.

The principle of the crossing method consists in mixing compatible mating types with complementary nutritional requirements and to plate this mixture on to minimal medium in order to select for diploid prototrophs. The reason for this rather complicated procedure is the very low frequency ($2 \cdot 10^{-4}$) of zygote formation which makes it almost impossible to recover diploids when using prototrophic strains. Ascus formation is induced only on a special sporulation medium. Thus in *Saccharomycopsis lipolytica* both phases, the haploid and the diploid are stable during vegetative propagation (Herman, 1971).

Staining Techniques. Nuclear material was stained either with a fluorescent stain (Acridin-Orange) according to the methods of Stock and Black (1970) or with Giemsa according to Sukroongreung and Miranda (1973). In order to achieve a better attachment of the yeast cells, the microscope slides were coated with egg albumin.

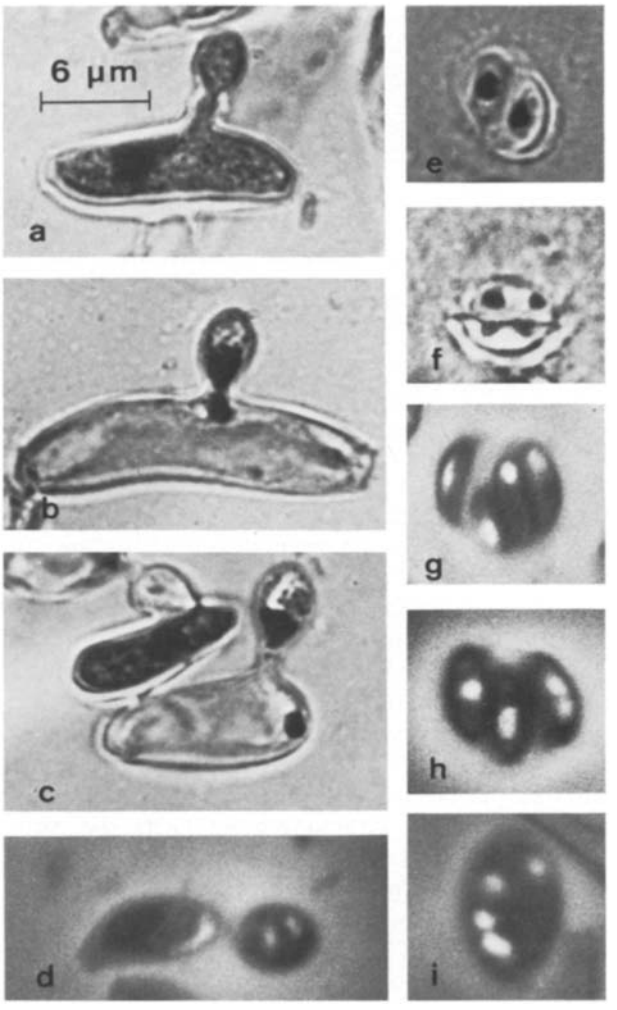

Fig. 1. Ascus development in *Saccharomycopsis lipolytica*. Nuclei appear dark after Giemsa staining or white after acridine orange fluorescent staining. **a** Diploid cell forming an ascus initial; **b** after a mitosis one nucleus migrates into the young ascus; **c** young ascus with diploid nucleus has separated from its mother cell by a cell wall; **d** meiosis I; **e** two spored ascus, spore wall formation after meiosisI; **f** meiosis II takes place within two young spores; **g** four spored ascus, spore wall formation after meiosis II; **h** three spored ascus, the two spores to the left have formed after meiosis II whereas the spore to the right has formed before meiosis II; **i** one spored ascus, spore wall formation before both meiotic divisions

Results and Discussion

1. Cytological Experiments

Diploid cells from *Saccharomycopsis lipolytica* resulting from the cross *+his lys*$^+$ × *−his*$^+$ *lys* were plated on sporulation medium. After 4–5 days, when ascus formation was completed, smear preparations were stained with Giemsa. The asci, which appear as lateral buds on the diploid cells, can be easily distinguished from the vegetative cells (Fig. 1 a–c). They were examined for the number of spores per ascus and for the number of nuclei in each spore (Table 1).

From the results presented in Table 1, one may make the following deductions:

1. As already described by other authors (see introduction), the two spored asci predominate of the other types of asci, those with four spores occur least frequently.

2. In each ascus four nuclei are always present. It can therefore be assumed that a normal meiotic division has taken place to give four haploid nuclei.