STUDY OF SOME ANOMALIES OF COPULATION IN *SACCHAROMYCES CEREVISIAE* HANSEN

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SUMMARY

A method for detecting mating-type mutations revealed the following phenomena in addition to point mutations for mating-type: (i) lethal deletions in the mating type chromosome, (ii) illegitimate copulation between cells of the same mating-type and (iii) plasmogamy without karyogamy.

1. INTRODUCTION

DURING an investigation of the genetic control of the sexual cycle of *Saccharomyces cerevisiae* Hansen, a method allowing the easy detection and isolation of mutants for mating type was used. This paper gives the results obtained from application of this method.

2. MATERIAL AND METHODS

(i) *Yeast strains and nomenclature*

The clones studied were obtained by selecting spontaneous mutants from the strain *Saccharomyces cerevisiae* Hansen no. 6153C9P1 named CP1 (obtained from Clavilier, 1962).

CP1 α *ade3-3 ade6 ural*
CP1-5 α *CYH-A* [ρ -(n)]
CP1-7 α *ade3-3 ade6 ural CYH-B*
CP1-4 α [ρ -(n)].

The gene *ade3-3* confers requirements for adenine and histidine. Reversions of the three auxotrophic markers were selected successively in the isolation of CP1-5 and CP1-4. Mutations for the resistance to the cycloheximide were selected by plating on medium containing 1 p.p.m. cycloheximide. The two mutations obtained are independent and named A and B. The "petite" strains were selected visually after plating on a glucose medium. The "petite" mutants selected were of the neutral type. For the genetic studies, we also used an *a*-mating type strain 4021D3P1 named DP1 (Clavilier, 1962) DP1 *a ade3-6 ade2 his1 trp1 met1*.

(ii) *Media*

YEG medium (yeast extract 0.5 per cent glucose 0.5 per cent) is used for stock maintenance, routine culturing and mating. The synthetic medium used, called medium GL, was described by Galzy and Slonimski (1957). This medium contains salts, vitamins and trace elements. The

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carbon substrate is lactic acid (5 g/l). In certain cases cycloheximide was added at a concentration of 1 p.p.m. (GL + CYH).

(iii) *Crossing technique*

Crosses were performed by mass mating according to the method of H. Jakob (1962) using high cellular densities (of the order of 10^8 to 10^9 cells per ml). Sporulation was obtained on the modified medium of Fowell (1952). Tetrads were analysed according to the technique of Johnson and Mortimer (1959).

(iv) *Selection of mating type mutants*

The method utilised in selecting mutants for the mating-type was based on the principle described by Pomper and Burkholder (1949) for the selection of prototrophs. In the present work, the two haploid clones plated together originated from the same strain; consequently they were fully isogenic except for a few markers, and had the same mating-type. The method

FIG. 1.—Principle of selection.

illustrated on fig. 1 has been previously described (Pellecuer, 1973). Theoretically, only zygotes having the dominant characters of the two clones should be able to grow and to yield colonies, and zygote formation will depend on mutation of mating type.

As indicated later, some of the selected clones were shown to arise through other routes than mutation of mating-type.

3. RESULTS

Two crosses were performed: CP1 $\times$ CP1-5 and CP1-7 $\times$ CP1-4. These four haploid clones are unable to grow on the selective media GL and GL + CYH; indeed multiple reversions are statistically unlikely and have never been observed in our experiments. The reversion of [rho-(n)] has also not been observed.

Table 1 gives the results obtained from these crosses. The two crosses performed only differ in the situation of the cycloheximide resistance in the parental strains. In both cases, some clones were obtained which were prototrophic and able to respire whatever the selective medium was. Only some of these clones were able to sporulate.

TABLE 1
Sporulation of selected matings

Cross	Selection medium	No. prototrophs tested		
		Total	Able to sporulate	Unable to sporulate
CP1 $\times$ CP1-5	GL	32	7	25
CP1-7 $\times$ CP1-4	GL	40	7	33
CP1-7 $\times$ CP1-4	GL + CYH	13	4	9

(i) *Study of the clones able to sporulate*

All the strains originating from a single sporulating colony had in common the following properties: prototrophy, normal respiration, inability to mate, cycloheximide resistance. This confirms that such strains really result from a cross of two cells from different clones, and that cycloheximide resistance is dominant in both crosses. Descendants of all these diploids were studied. Some of them yielded four viable spores (2 α and 2 a) in each ascus. Such was the case for 2/7 strains obtained from the cross CP1 $\times$ CP1-5, for 4/7 strains resulting from the cross CP1-7 $\times$ CP1-4 and selected on medium GL, and for four strains obtained from the same cross but selected on medium GL + CYH.

From each strain, 10 to 25 asci were dissected. The segregation of markers was normal: *i.e.* of monogenic type for histidine and uracil, and of digenic type for adenine. The segregation of cycloheximide resistance was also of monogenic type, as was segregation of mating type, both in the original selected diploids and in backcrosses.

Two conclusions can be drawn:

- The "cycloheximide resistance" characters are monogenic. Different loci are involved in the two mutations *CYH A* and *CYH B*.
- The gene *a* found in the descendants did not pre-exist in the parents.

It is evidently due to spontaneous mutation from α to a . Such mutation has been observed by Leupold (1950) in *Schizosaccharomyces pombe* and by Ahmad (1952), Roman and Sands (1953) and Hawthorne (1963) for *Saccharomyces cerevisiae*.

The eight other strains able to sporulate exhibited only two viable spores per ascus. The two other spores germinated, yielded a few buds on the microdissection plate, but then ceased to grow. For 7 to 11 asci of each of these strains, all the spores obtained were studied. The results are given in table 2. Segregation is statistically monogenic for cycloheximide resistance and for the uracil and histidine requirements; it is digenic for the adenine requirement. This suggests that a cross between the two clones occurred. However, all the spores able to develop were of α mating-type; consequently a lethal character is associated with the a mating-type.

In conclusion it seems that the mating type changes involved lethal mutations, deletions including some or all of the cistrons governing the α character. Such deletions were described by Hawthorne (1963).

TABLE 2
Segregation of markers of diploids giving only 2 viable spores

Cross	No. clones	No. Asci	Segregation of markers (+/-)				
			a/α	<i>ade</i>	<i>his</i>	<i>ura</i>	CYH
CP1 $\times$ CP1-5	5	49	0 : 98	24 : 64	47 : 51	47 : 51	50 : 48
CP1-7 $\times$ CP1-4	3	28	0 : 56	17 : 39	33 : 23	30 : 26	25 : 31

(ii) *Study of the clones unable to sporulate*

All the clones unable to sporulate are prototrophic, have normal respiration and possess the α phenotype.

(a) *Clones from CP1 $\times$ CP1-5.* Twenty-five clones exhibited cycloheximide resistance. One clone taken at random has been more particularly studied. It was crossed with strain DP1 of a -mating-type and 36 asci obtained after sporulation were subjected to microdissection. The viability of the spores was very poor, about 10 per cent. Three asci with four viable spores exhibited an irregular segregation for the markers and yielded some cultures auxotrophic for uracil and some able to sporulate. All these observations are consistent with triploidy of the clones, implying diploidy of one parental strain. Such diploids would result from an "illegitimate" copulation between two cells of the same mating type, giving a diploid α/α unable to sporulate.

(b) *Clones from CP1-7 $\times$ CP1-4.* Nine clones selected on medium GL + CYH were naturally cycloheximide resistant. Two clones were more thoroughly studied. After crossing with strain DP1 of a -mating type 45 asci were dissected in one case and 27 in the other. The viability of spores was poor; only three asci with four viable spores were obtained from each cross. The presence in the segregation products of cultures auxotrophic for uracil and cultures able to sporulate is evidence that here too the clones were triploid, implying that one parental strain was a diploid α/α .

Among 33 clones selected on medium GL from the cross CP1-7 $\times$ CP1-4, 28 were cycloheximide resistant whereas five are sensitive. From four resistant clones, the asci obtained by sporulation after crossing with DP1 were dissected. The viability of the spores was very poor and the segregation of markers was similar to that described above. These four strains would be diploids α/α as well. The five cycloheximide sensitive clones were all studied in detail. Asci obtained after crossing to DP1 showed high spore viability (95 per cent). For each clone, the number of asci studied was as follows:

Strain 552	15 asci
553	26 asci
554	17 asci
555	23 asci
556	16 asci

The segregation of the markers was exactly the same for all the asci: mating type $2a/2\alpha$, methionine $2+/2-$, tryptophan $2+/2-$, uracil $4+/0-$, cycloheximide $0+/4-$. Thus the five cycloheximide sensitive clones are prototrophic for adenine, histidine and uracil, they are able to respire normally and they can participate in crosses with an a -mating type strain and form typical diploids showing normal segregation. These properties show them to be haploid of α -mating type, and they look very much like CP1-4, except for the respiratory deficiency. However, no reversion with respect to respiratory deficiency was obtained when 10^9 CP1-4 cells were plated onto a GL medium.

It is possible that a copulation had occurred between two cells of the same mating type which, however, were unable to form a genuine diploid, but only a dikaryon which segregated on budding. A similar phenomenon has been reported by Wright and Lederberg (1957). In the cross CP1-7 $\times$ CP1-4, prototrophy and cycloheximide resistance are contributed by different parents. Buds formed by dikaryotic zygotes on medium GL + CYH are not viable because they are either cycloheximide-sensitive or auxotrophic. On cycloheximide-free medium GL, on the other hand, the prototrophic, cycloheximide sensitive component could be recovered in buds, but not the auxotrophic component.

4. DISCUSSION

Certain of the phenomena described here are classical and have already been described and schematised (Fowell, 1969):

(a) Spontaneous mutations of the mating-type gene were observed in all the crosses.

(b) Deletions of part of the sexual chromosome were observed, allowing the cell to behave as one of the opposite mating-type.

(c) "Illegitimate" copulations between cells of the same mating-type yielded diploids which were homozygous for mating-type (α/α) and unable to sporulate.

A phenomenon which is not so well known was also revealed: the possibility of plasmogamy followed by a budding of haploid cells without karyogamy. It would be interesting to determine whether such plasmogamy happens only between cells of the same mating-type or whether it can also

occur between cells of opposite mating-types. Whether the dikaryotic state can be maintained or can only exist temporarily in the zygote should also be determined. The phenomenon might be an excellent working tool in mitochondria genetics, since it permits mingling and segregation of mitochondria without involvement of the nuclei.

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