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Induction of cytoplasmic respiratory deficiency in yeast by phenethyl alcohol

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1. INTRODUCTION

It is claimed that phenethyl alcohol (PEA) inhibits replication of the bacterial chromosome by preventing the initiation of DNA synthesis (Treick & Konetzka, 1964; Lark & Lark, 1966). Other investigators have concluded that PEA primarily affects RNA metabolism both in bacteria and in mammalian cells and that inhibition of DNA synthesis is a secondary effect (Rosenkranz, Mednis, Marks & Rose, 1967). The synthesis of mitochondrial DNA (MDNA) in yeast cells can be specifically affected by certain mutagens, notably acridine dyes, resulting in the petite or ρ-mutation. Cells in this condition synthesize non-functional mitochondria lacking cytochromes *a* and *b* and transmit the deficiency to daughter cells during vegetative growth. Reversion to normal has not been seen and the situation constitutes a classic case of cytoplasmic inheritance.

Mounolou, Jakob & Slonimski (1968) have examined several petite strains and shown that their MDNA has been grossly altered although not physically lost. Earlier reports of loss of MDNA in petites were found to be inconclusive because the quantity of MDNA is generally much reduced by the conditions of glucose repression that were used in these experiments. (For review of the yeast mitochondrial system see Roodyn & Wilkie, 1968.) However, it is widely believed that the petite condition results from an effective loss of MDNA as a functional genetic unit, a conclusion supported by the apparent irreversible nature of the mutation.

Experiments are described here in which PEA is shown to induce the petite mutation. Since PEA has no known mutagenic action it is reasonable to discuss petite induction by this substance in terms of its possible effect on MDNA replication.

2. MATERIALS AND METHODS

Yeast strains were taken from the collection in the authors' laboratory with various nuclear markers as listed in Table 1. A 1% yeast-extract, 2% peptone medium (YEP) was used with one or other of the carbon sources glucose (2%), glycerol (4%), melibiose (4%) and galactose (4%).

In initial tests to establish the ρ-condition, petite-determining medium (PDM) was used in which YEP-glycerol is supplemented with 0·2% glucose. This allows a limited amount of colony development from ρ-cells as against no growth on the non-fermentable substrate alone. PEA (Eastman Chemical Co.) was added directly to sterilized media in the amounts indicated and in the case of agar media, just prior to pouring. Inhibitory effects of PEA on the various strains was determined by the drop-out method, that is, by placing replica drops of cell suspensions on the agar surface at appropriate spots in a series of plates. Each drop usually contained about 10^4 cells. Cells were also plated out in some cases and platings of about 100 cells per plate were generally used.

Micromanipulation of spore tetrads was carried out using the De Fonbrune micromanipulator. For details of sporulation and crossing see Hawthorne & Mortimer (1960).

Anaerobic culture was effected in a Fildes cylinder under nitrogen.

Table 1. *Growth inhibition and petite induction by PEA*

Strain	Genotype*	Growth on PEA 0·1 % S†	Growth on PEA 0·1 % G	Growth on PEA 0·25 % S	Growth on PEA 0·25 % G	Percentage petite (nearest whole no.) Control	Percentage petite (nearest whole no.) PEA-grown
Haploids							
617	a ad_6	+ +	+	+	−	15	30
82	α le ly me	+ +	+ +	+	+	1	25
45	a hi ur	+ +	+ +	−	+	1	5
41	α hi	+ +	+ +	−	+	1	13
26	α ad_2	+ +	+ +	−	+	1	75
19	α hi le	+	−	−	−	1	40
11	a ad_2	+ +	+ +	+	−	2	60
Diploids and progeny							
Diploid 1 (82 × 617)		.	.	+	−	2	10
Tetrad							
1–1A	le ly me	.	.	+	−	.	66
1–1B	me	.	.	+ +	+ +	.	
1–1C	ad_6	.	.	+ +	+ +	.	
1–1D	ad_6 ly le	.	.	+	−	1	95
Random spores							
1–1	ad_6	.	.	+ +	+	1	40
1–2	le ly me	.	.	+	−	1	70
1–3	ad_6 le ly	.	.	+	−	1	30
1–4	ad_6 me	.	.	+	−	1	40
1–5	ad_6 le ly	.	.	+	−	2	30
Diploid 2 (26 × 1–1D)							
Tetrad							
2–1A	.	+ +	−	−	−	.	50
2–1B	.	+	+	−	−	.	2
2–1C	.	+ +	−	+	−	.	60
2–1D	.	+	−	−	−	.	10

* For symbols see Hawthorne & Mortimer (1960).

† S, YEP-glucose agar; G, YEP-glycerol agar.

+ +, growth as in controls; +, much reduced amount of growth; −, no growth.

3. RESULTS

Growth inhibition

The most important feature of *Saccharomyces cerevisiae* in mitochondrial studies is that, being a facultative anaerobe, the organism can grow and divide without a functional respiratory system, provided there is fermentable substrate available. Specific inhibition of the mitochondrial system is thus detectable as a failure to grow on the non-fermentable substrate (glycerol) in the presence of the inhibitor, while growth would be unaffected on a glucose-containing medium. A number of strains were tested in this way for inhibition of growth in the presence of PEA both on fermentable and non-fermentable medium. It was found that the patterns of growth and induction on melibiose medium with PEA resembled those found on glucose, while inhibition on galactose resembled the pattern found on glycerol. Table 1 lists results on glucose and glycerol.

At 0·1 % PEA, out of 11 strains tested, six grew well on fermentable and non-fermentable substrate. The remaining five did not grow on glycerol and three of these grew only poorly on glucose.

At 0·25 % PEA, out of 21 strains tested four grew on both substrates, and four failed to grow on either substrate; three of these latter strains had shown only poor growth on 0·1 % PEA. Ten strains grew only on glucose at 0·25 % PEA while three strains grew only on glycerol at this concentration.

At 0·5 % PEA, all strains were totally inhibited on both media. Thus PEA shows general toxicity; only over a fairly narrow range of concentration does it specifically inhibit growth on the non-fermentable substrate, while three strains actually show preferential growth on glycerol.

Induction of petite

Cells were sampled for petite frequency from drop-out colonies on the 0·1 % PEA-glucose plates. There was a significant increase in petite frequency in all cases (Table 1). That the increase was due to induction rather than selection of petite cells on glucose was checked for strain 11 by plating out individual cells on to 0·1 % PEA-glucose medium. Approximately 20 % of the individual colonies on the PEA were petite, compared to 1 % on the control plates. On 0·1 % PEA-glycerol medium, the frequency of petite cells in the drop-out colonies was also increased (Table 2). Glycerol medium specifically selects against the growth of petite cells, so that these could only have increased their frequency by induction during the growth of respiratory competent cells.

Table 2. *Induction of petite by 0·1% PEA on non-fermentable (G) medium.*

	Percentage petite	
Strain	Control	PEA-grown
82	4	23
45	1	17
41	6	15
11	2	20

Petite frequency was also investigated in cells growing under anaerobic conditions, which repressed mitochondrial synthesis (see Marchant & Smith, 1968). Little difference could be seen in petite induction, compared to aerobic growth on glucose (Table 3).

Thus PEA induces petite whether the yeast is actively respiring as on glycerol, respiring but glucose-repressed, or anaerobically fermenting.

Table 3. *Induction of petite by 0·1% PEA in aerobic and anaerobic culture on fermentable (S) medium.*

	Percentage petite	
Strain	Aerobic	Anaerobic
82	15	25
45	15	15
19	50	30
11	60	70
2–1A	35	50
2–1D	65	70

Confirmatory tests for petite mutation

One of the effects of PEA was to produce small, white colonies on glucose, which being initially unable to grow on glycerol were mistaken for petite mutants. However, after several weeks, these colonies eventually supported vigorous growth on glycerol. To avoid confusion, petite colonies were eventually scored only after 2 weeks on PDM, rather than after the usual 4 days.

In a number of cases petites were subjected to genetic tests to prove the cytoplasmic basis of the mutation. Petites were isolated on PDM from strains 617, 1–1 D, 1–1 A, 1–1, 1–4, and 2–1 A. Each was crossed with $\rho+$, either from strain 41 or strain 45 depending on mating type; in every case the resulting diploid was $\rho+$, and so were all of the resulting tetrads that were analysed. When the isolates were crossed, on the other hand, to known $\rho-$ mutants derived either spontaneously or by acriflavine treatment of 41 and 45, all the derived diploids were petite. Finally, two of the PEA-induced petite strains were crossed and the resulting diploid was petite. These results provided evidence that the PEA-induced petites were $\rho-$.

Cytochrome absorption spectra of six PEA-induced petites (of 1–1 D, 26 and Tetrad 2–1 A, B, C, D) grown on the non-repressing sugar melibiose, failed to reveal cytochromes *a* and *b*, while cytochrome *c* was slightly enhanced compared to the $\rho+$ parent strains. This is characteristically a petite phenotype.

4. DISCUSSION

This investigation began with the supposition that, in view of the known effect of PEA in preventing DNA replication in bacterial and mammalian cells, this substance might act on yeast in a similar way and by preferentially inhibiting the synthesis of mitochondrial DNA would give rise to the petite mutation. The present work confirms that PEA can induce cytoplasmic respiratory deficiency in various strains of *S. cerevisiae*. It remains to be established whether PEA acts by preventing the replication of mitochondrial DNA or by some other mechanism.

The patterns of growth inhibition on fermentable and non-fermentable substrates indicate that PEA may have a complex action. On the one hand, there is the predominant tendency for growth to be inhibited on glycerol and the occasional appearance of small, white $\rho+$ colonies on glucose, which suggests an attack on the respiratory system. On the other hand, there is only a narrow margin between the concentration range which inhibits respiration (0·1 %–0·25 %) and the range which inhibits growth generally (0·25–0·5 %). These results are somewhat analogous to those obtained by Jacob, Brenner & Cuzin (1963), and Lark & Lark (1966), on bacteria, in so far as both reports mention a rather delicate optimum concentration of about 0·25 % above which PEA has lethal effects.

No correlation was found between inhibition of respiration and induction of petite. Some strains showed increased petite frequency while growing well on glycerol at 0·1 % PEA (Table 2). At the other extreme, PEA is equally effective under anaerobic conditions (Table 3). Thus it it possible that PEA induces petite by one mechanism and inhibits respiration by another. For instance, Terenzi & Storck (1968), who report the inhibition of respiration by PEA in *Mucor rouxii*, suggest that PEA may adversely modify mitochondrial permeability.

An electron microscope study on PEA-induced petites from 26, 1–1 D and their tetrad 2–1 A, B, C, D (Smith, Marchant, Maroudas & Wilkie, 1968) has revealed the presence of mitochondria similar to those found in acriflavine or u.v.-induced petites of the same strain. Thus, if acriflavine causes gross alteration of the mitochondrial DNA, as suggested by the work of Mounolou, Jakob & Slonimski, while PEA causes loss, the net effect would appear to be indistinguishable on the structural level.

SUMMARY

Phenethyl alcohol is shown to induce cytoplasmic respiratory deficiency (petite mutation) in various strains of *Saccharomyces cerevisiae*. Apart from petite induction, phenethyl alcohol also affects cell growth both in respirable and non-respirable media. This sensitivity to growth inhibition appears to be complex, and does not correlate with the degree of induction of respiratory deficiency.

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