ANAEROBIC GROWTH AND FORMATION OF RESPIRATION-DEFICIENT MUTANTS OF VARIOUS SPECIES OF YEASTS

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1. Introduction

Yeast *Saccharomyces cerevisiae* has often been used in the study of biogenesis and function of mitochondria taking advantage of the abilities of this species to grow under anaerobic conditions and to give rise to cytoplasmic respiration-deficient mutants. It has been usually assumed that both abilities are related to the high fermentation capacity of this yeast which can furnish energy for growth under conditions when oxidative phosphorylation is not functioning. However, this assumption has not yet been subjected to unequivocal experimental tests. Little is known of how other yeast species behave in this respect except that many species of 'petite negative' yeasts do not form cytoplasmic respiration-deficient mutants [1,2]. The use of other yeast species may help to clarify this problem and also provide new experimental possibilities for the study of mitochondria not encountered with the common baker's or brewer's yeasts.

By employing a number of different yeast species this paper shows that the ability of a cell to grow under strictly anaerobic conditions is not related to its respiration or fermentation capacities and that these capacities are also not directly responsible for the ability of some species to form respiration-deficient mutants.

2. Experimental

The following yeast species were obtained from the yeast collection of the Chemical Institute of the Slovak Academy of Sciences, Bratislava: *Bretanomyces anomalus* (31-2-1), *Saccharomyces lactis* (21-3-1), *Fabospora fragilis* (51-1-1), *Zygosaccharomyces fermentati* (35-8-2), *Candida parapsilosis* var. intermedia (29-20-10), *Endomyces magnusii* (42-1-1), *Schizosaccharomyces pombe* (44-1-3), and *Schizosaccharomyces versatilis* (44-3-1). They were cultured in a semi-synthetic medium [3] with glucose, glycerol or ethanol as carbon source on a reciprocal shaker at 30°C. Cells precultured for 24 hr statically in test tubes were used for inoculation. For aerobic growth, the culture flasks were filled to one tenth of their volume with the medium and plugged with cotton. Growth under strictly anaerobic conditions was secured by culturing cells in a flask filled with medium almost to the neck and equipped with a mercury trap; the medium in the flask was flushed with oxygen-free nitrogen or argon for 20 min both before and after inoculation. The medium for anaerobic growth was supplemented with 0.26% Tween 80 and 0.001 2% ergosterol; when no growth occurred, the medium was enriched with 0.2% flax oil to provide linolic and linolenic acids, but this failed to induce growth.

Growth was followed by counting the cells in a haemocytometer and by plating on solid agar medium. Respiration-deficient mutants were detected by differential plating on glucose and glycerol media and by the tetrazolium overlay method [4]. Fermentation and respiration of cells, grown aerobically on glucose up to stationary phase, were determined by the conventional manometric technique. Cytochrome spectra

were measured in the Hitachi–Perkin-Elmer 356 spectrophotometer. Mitochondria were isolated and their ATPase determined by the published procedures [3].

3. Results and discussion

Basic growth and metabolic characteristics of the selected yeast species are listed in table 1.

The species can be grouped into several overlapping groups: In some species (*S. lactis, Z. fermentati, Sch. pombe; F. fragilis* was similar to *S. lactis* in all the properties examined), antimycin A inhibited respiration completely and reduced the aerobic growth yield on glucose to an anaerobic level but did not arrest growth entirely. This indicated that the less efficient source of energy, i.e. fermentation, could supply enough energy to support aerobic growth on these non-respiring cells. Yet, none of these species was able to grow under strictly anaerobic conditions.

On the contrary, *B. anomalus,* which was able to

Table 1

Fermentation, respiration and growth of selected yeasts. o and + mean respectively the absence or presence of the component or function

Characteristics \ Species	*B. anomalus*	*S. lactis*	*Z. fermentati*	*C. parapsilosis*	*E. magnusii*	*Sch. pombe*	*Sch. versatilis*
Anaerobic growth	+	0	0	0	0	0	+
Aerobic growth							
Growth yield (10^6 cells/ml) in the stationary phase on 0.5% glucose in the presence of the following inhibitors:							
None	232	404	190	400	230	36	8
Ethidium bromide (25 μM)	55	104	37	200	1	24	8
Antimycin A (2 μg/ml)	74	90	46	240	< 1	28	3
Growth on 2% ethanol or glycerol	+	+	+	+	+	0	0
Cytochromes	a,b,c	a,b,c	a,b,c	a,b,c	a,b,c	a,b,c	0
Fermentation (μl/hr/mg dry weight) $Q^{N_2}_{CO_2}$	20	218	66	46	42	402	194
Respiration (μl/hr/mg d.w.)							
Q_{O_2}	78	134	66	138	43	10	0
Q_{CO_2}	99	185	102	202	52	404	192
Inhibition (%) by antimycin A (2 μg/ml)	92	100	100	27	68	100	–
Formation of respiration-deficient mutants by:							
Acriflavin (67 μM)	+	0	0	0	0	?*	Not decided
Ethidium bromide (25 μM)	+	Lethal**	Lethal**	0	0	?*	Not decided

* The culture grown with acriflavin or ethidium plated on solid glucose medium gave rise to viable very slowly growing cells. The cells from a colony were grown in liquid culture medium and were found to lack cytochromes *a* and *b*.

** The culture grown with ethidium plated on solid glucose medium did not give rise to any colonies.

grow anaerobically, exhibited an unusually low fermentation capacity. Thus, the ability of yeasts to grow in the absence of molecular oxygen is not directly related to their fermentation capacity. Neither could the inability to grow anaerobically be explained by some complex nutritional requirements, since the enrichment of growth media with yeast extract or with flax oil did not change the growth patterns. Molecular oxygen may be required in some species to establish an appropriate redox state in the cells (for instance $NAD/NADH_2$ or –SH/ –SS– ratios) necessary for growth supported by fermentation.

E. magnusii was not able to grow in the presence of antimycin A indicating that the fermentation energy was not sufficient to support aerobic growth of this species. This is remarkable both because the fermentation rate of *E. magnusii* was still higher than that of *B. anomalus* which thrived anaerobically and because the inhibition of respiration by antimycin A was not complete. It seems that the antimycin A-insensitive respiration could not furnish energy for growth.

On the other hand, *C. parapsilosis* with a fermentation rate as low as *E. magnusii* and with respiration which was also only partly inhibited by antimycin A could grow aerobically on glucose in the presence of antimycin A. It is conceivable that in this species an antimycin A-insensitive pathway may provide energy necessary for growth.

In confirmation of some reports [1,5], cells of *Sch. versatilis* were found to be non-respiring and cytochrome-deficient. A possibility may be raised that *S. versatilis* is a cytoplasmic respiration-deficient (ρ^-) mutant derived from some respiration-sufficient strain. However, contrary to ρ^- mutants which have lost oligomycin-sensitivity of the mitochondrial ATPase [6,7], the ATPase of isolated mitochondria from *Sch. versatilis* was found to be inhibited by 86% with 40μg/ml oligomycin, the extent of inhibition corresponding to that previously found with wild-type *S. cerevisiae* [3]. If other mitochondrial properties, known to be impaired in ρ^- mitochondria, are preserved in *Sch. versatilis*, the species may become an organism of choice for the study of mitochondrial functions not related to respiration.

In accord with Bulder's classification [1], only *B. anomalus* was found to be a 'petite-negative' species giving rise to viable ρ^- mutants. The present results indicate that the group of 'petite negative' species should be divided in at least two subgroups: In the first (*C. parpsilosis, E. magnusii*) (for *C. parapsilosis* see also ref. [8]), ethidium bromide and acriflavin could inhibit transcription of mitochondrial DNA (as reflected by lowering of growth yield on glucose and arrest of growth on ethanol), but no evidence for mutant formation was obtained. In this respect, these species resemble animal cells. In the second subgroup (*S. lactis, F. fragilis, Z. fermentati*), acriflavin was not mutagenic but ethidium bromide did apparently cause lethal mutations (cf. ref. [9] for additional data on *S. lactis*). It is clear from the data of table 1 that the lethality could not be due to insufficient fermentation capacity of these strains as is usually assumed, but must have some other, yet unknown, reason. Slowly-growing cytochrome-deficient cells, formed by *Sch. pombe* grown with ethidium, may represent nuclear respiration-deficient mutants as shown and discussed by Heslot et al. [10]. However, owing to the high frequency at which these slowly-growing variants were formed, the cytoplasmic nature of some of them cannot be excluded.

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