STRUCTURAL DIFFERENTIATION OF OBLIGATELY AEROBIC AND FACULTATIVELY ANAEROBIC YEASTS

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INTRODUCTION

Although biochemical criteria are used in the taxonomic differentiation of the yeasts, the cytologist has generally considered that the structures of these organisms are essentially alike, although more readily discernible in some than in others. Actually, the fine structures of different species of yeast cells vary, particularly with respect to membrane systems and mitochondria, according to their relative capacities to respire or ferment under aerobic conditions.

Based upon their requirements for molecular oxygen, the yeasts are divided into obligate aerobes and facultative anaerobes. The latter group has been subdivided into "petite positive" and "petite negative" types, according to differences in their inducibility to produce respiratory deficient mutants upon treatment with acriflavine or euflavine (1, 2, 4). Although certain respiratory characteristics of the two types of yeasts were determined in these studies, they were not correlated with structural features of the cells.

Several articles have dealt with the influence of anaerobic conditions (10, 11, 21), glucose concentrations (5, 10, 17, 22), nonfermentable catabolites (17), or less readily fermentable sugars (10) on mitochondrial development, but these articles have been confined essentially to *Saccharomyces cerevisiae*, whose versatility to respire or ferment a variety of different substrates under various physical conditions is not characteristic of yeasts in general. Although *Candida* (*Torulopsis*) *utilis* was used in an investigation of the origin of mitochondria in yeasts (11), its use was later abandoned, in favor of *S. cerevisiae*, with the statement that experimentation with this organism (*C. utilis*) had proved difficult, in that its cytological characteristics had varied greatly from one experiment to another (21). The earlier study of *C. utilis* (11) had revealed a complex, reticular membrane system in electron micrographs of anaerobically grown cells, while the later electron micrographs of anaerobically grown cells of *S. cerevisiae* (21) revealed no such membrane system, the cytoplasm being essentially devoid of morphologically demonstrable mitochondria and other membrane systems.

The wide diversity of physiological characteristics found among the various species of yeasts of similar gross morphology and simple cultural requirements offers an ideal opportunity for relating functional and structural differences in individual cell types. This study was undertaken to investigate differences, rather than similarities, in yeasts which are known to represent three physiological and genetic categories, namely (*a*) obligate aerobes, (*b*) petite positive, facultative anaerobes, and (*c*) petite negative, facultative anaerobes.

MATERIALS AND METHODS

Because of their diversity of respiratory and genetic characteristics the following were chosen for this study: *Rhodotorula gracilis* NRRL Y1091, a nonfermenting, obligate aerobe (12); *Saccharomyces* "Carbondale" culture B8502, a petite positive, facultative anaerobe; *Saccharomyces fragilis* NRRL Y665, a facultative anaerobe, noninducible by euflavine to produce respiratory-deficient mutants (4), or inducible by acriflavine to produce occasional, aberrant petites (1); and *Candida utilis*, a facultative anaerobe not inducible by euflavine or acriflavine to produce respiratory-deficient mutants (1, 4). The above biochemical and genetic characteristics of each were thoroughly verified, preceding cytological studies of these organisms.

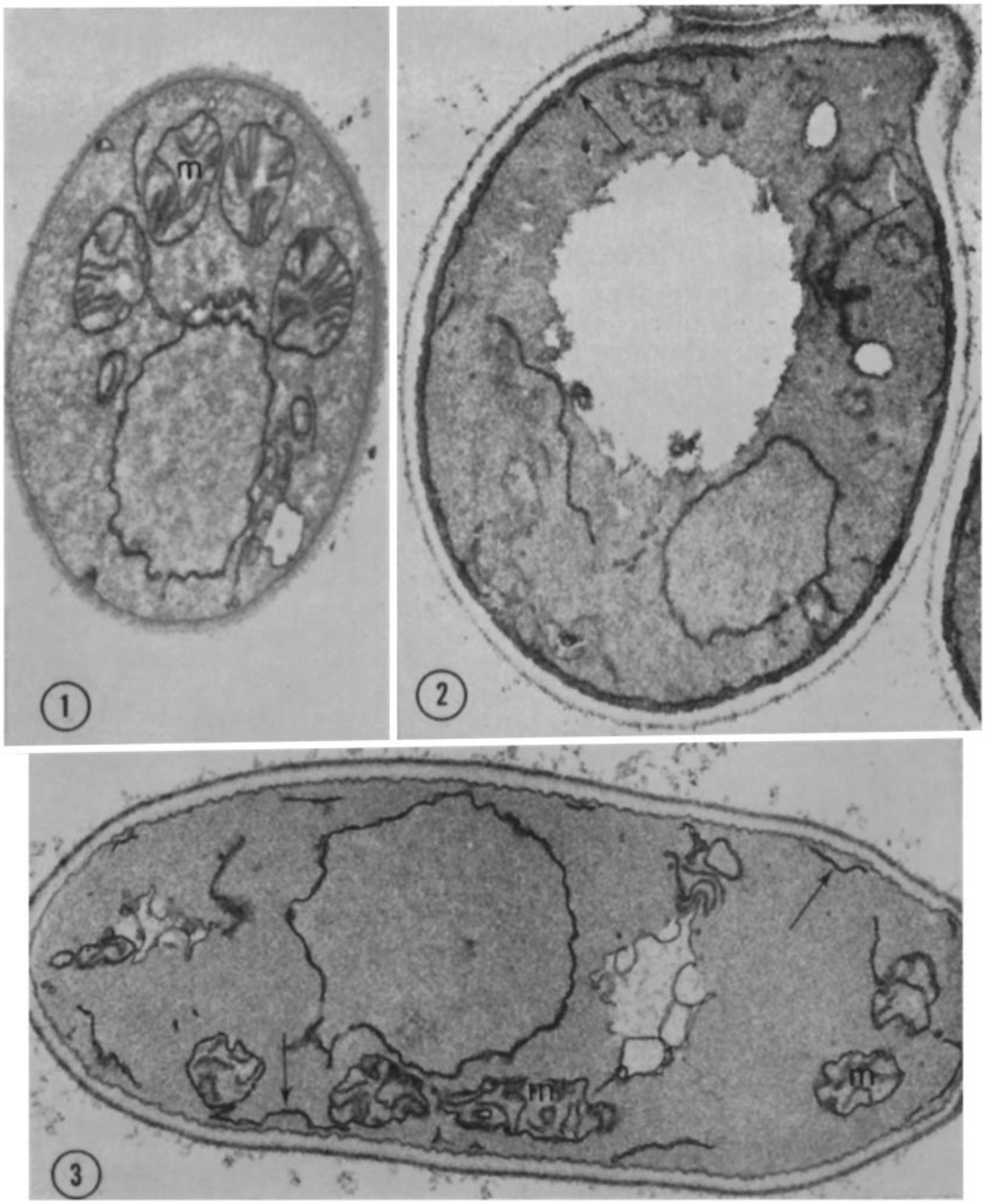

FIGURE 1 Logarithmic growth phase cell of *Rhodoturula gracilis* grown in 2% glucose–yeast extract–salts medium. The mitochondria (*m*) are typical, cristate structures. × 32,000.

FIGURE 2 Logarithmic growth phase cell of *Saccharomyces* Carbondale strain B8502 grown in 2% glucose–yeast extract–salts medium. Mitochondria are not conspicuous in these cells. A broken, or intermittent membrane (arrows) lies adjacent to the plasma membrane and, in some regions, extends out into the cytoplasm. Note that no such membrane exists in the obligate aerobe, *R. gracilis*. × 32,000.

FIGURE 3 Logarithmic growth phase cell of *Saccharomyces fragilis* grown under the previously described conditions. Numerous, typical mitochondria (*m*) appear in this non-aerobic fermenter and only vestiges of the intermittent membrane (arrows), which is a prominent feature of the highly fermentative *Saccharomyces* B8502, are seen. × 32,000.

The yeasts were grown in glucose–yeast extract–salts medium (13). To insure identical culture conditions for all the yeasts, cells from agar slants of the medium were used to inoculate 10 ml quantities of the broth medium in 250 ml Erlenmeyer flasks and shaken on a reciprocal shaker for 24 hr at 30°C. These cultures were used to inoculate 200 ml of the medium in 1 liter Erlenmeyer flasks. The flasks were incubated at 30°C on a rotary shaker, and samples were taken at 2-hour intervals to measure growth, as indicated by turbidity with a Klett-Summerson colorimeter equipped with a 420 μm filter. When plots of the turbidity readings showed that the cultures were well within the exponential growth phase, samples were removed, washed twice in distilled water, fixed in 2% $KMnO_4$ for 4 hr at 4°C, washed three times with water, stained with uranyl acetate, dehydrated in a graded alcohol series, and embedded in methacrylate. Sections were cut with a Porter-Blum microtome equipped with a diamond knife, and attached to Formvar-coated grids. Some of the sections were stained with lead citrate, and all were examined and photographed with an RCA EML electron microscope. For some experiments with the Carbondale culture, *Saccharomyces* B8502, 1% sodium lactate was substituted for the fermentable glucose in the growth medium, and cells from logarithmic growth were prepared for electron microscopy in the manner described above.

The respiratory activities of the yeasts were determined by conventional Warburg manometric methods. Cells from cultures in exponential phase were washed and suspended in 0.5% glucose in $\frac{1}{15}$ M KH_2PO_4. Respiratory quotients (RQs) were calculated from the μl O_2 and μl CO_2 measured at 15-min intervals.

RESULTS

Electron Microscopy of the Cells

A section of a cell of *R. gracilis* taken during logarithmic growth in 2% glucose medium is shown in Fig. 1. The distinctive features of this cell are the large, well-developed, cristate mitochondria. Shown in Fig. 2 is a section through a logarithmic phase cell of *Saccharomyces* Carbondale culture B8502 grown under the same conditions. Although various membranes and vesicles are visible, mitochondria are rudimentary and in-

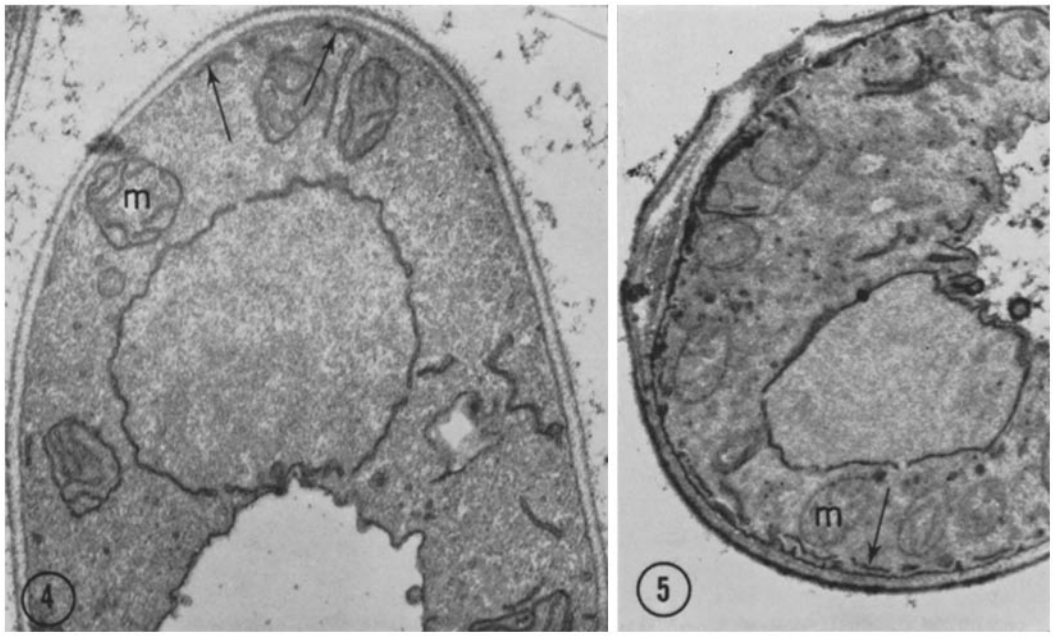

FIGURE 4 Logarithmic growth phase cell of *Candida utilis* grown as previously described. The features of this culture are essentially like those of *S. fragilis*. × 32,000.

FIGURE 5 Logarithmic growth phase cell of *Saccharomyces* Carbondale strain B8502 with sodium lactate substituted for glucose in the medium. Under these conditions well-developed, cristate mitochondria (*m*) appear in appreciable numbers. The intermittent membrane (arrow) is clearly defined in these cells. × 32,000.

conspicuous. A structural feature, just beneath the cytoplasmic membrane of this highly facultative anaerobe, which is not observed in the obligately aerobic, *R. gracilis*, is a discontinuous reticular membrane. In Figs. 3 and 4, respectively, are shown sections of logarithmic phase cells of the two other facultatively anaerobic yeasts, *S. fragilis* and *C. utilis*. These figures are very similar to each other and, with respect to the rather well-developed mitochondria, resemble that of *R. gracilis*. Small fragments of other membranes are arranged parallel to the plasma membrane, as seen in the highly fermentative, Carbondale *Saccharomyces*, but to a much less extent than in that organism. Fig. 5 shows a cross-section through a logarithmic phase cell of Carbondale *Saccharomyces*, grown under the conditions described above, except that 1% sodium lactate was substituted for glucose in the medium. Under this strictly aerobic condition, as Schatz (17) has shown in *S. cerevisiae*, this culture also produces morphologically well-developed mitochondria. The discontinuous membrane in the peripheral region of these cells is retained, indicating that its existence does not depend upon the fermentable substrate.

TABLE I

Aerobic RQ and Mitochondrial Integrity of Four Species of Yeasts during Logarithmic Growth in 2% Glucose Medium and Their Inducibility by Acriflavine to Produce Petite Mutations

Culture	Aerobic RQ	Well-formed mitochondria	Acriflavine induced petites
S. Carbondale B8502	4.3	—	+
S. fragilis	1.1	+	—
C. utilis	1.1	+	—
R. gracilis	1.2	+	N.D.*

* Not done. *R. gracilis* is an obligate aerobe. It is extremely unlikely that an agent could induce aerobic independence and respiratory deficiency in an organism at the same time.

The Respiratory Activities of the Cells

The relationships among the respective RQs of the cultures, their formation of morphologically well-developed, cristate mitochondria, and their inducibility to petite formation by acriflavine are shown in Table I. Logarithmic phase cells of *Saccharomyces*, harvested from 2% glucose under aerobic conditions, ferments with an RQ greater than 4.0, while *C. utilis* and *S. fragilis*, although both are producers of gas under the reduced oxygen tension of a fermentation tube, simply respire, as indicated by their average RQs of 1.1. *R. gracilis* does not grow anaerobically and fails to produce gas from glucose broth in fermentation tubes. Respirometer tests with this organism, like those described above, revealed an RQ of 1.2.

DISCUSSION

It is clear from the reports of others (5, 10, 17, 22) who used *S. cerevisiae*, and from our results with the Carbondale *Saccharomyces* culture, that glucose concentrations as low as 2% repress the formation of mitochondria, as well as respiration, in the highly fermentative bakers' yeasts, i.e., those which respire with a high RQ even under highly aerobic conditions. However these effects are not apparent in the obligate aerobes which assimilate glucose by the oxidative mechanism and regularly exhibit the most highly morphologically developed mitochondria. Between these two extreme groups are many species, as exemplified by *S. fragilis* and *C. utilis*, which ferment, or produce gas, vigorously in the reduced oxygen tension of a Durham or Smith fermentation tube and in aerated culture show little if any glucose repression, either on respiration or on the morphological development of mitochondria. This study, though limited to but few species, suggests that the species which ferment glucose under highly aerobic conditions with a high RQ and possess poorly defined mitochondria under these conditions fall into the group which DeDeken (4) and Bulder (1) found inducible by euflavine and acriflavine to form viable respiratory-deficient mutants, while those which respire and possess well-developed mitochondria under these conditions are rarely induced by these agents to form the viable mutants.

The work of Slonimski (18) and Bulder (2) offers an explanation for the differences in petite inducibility in yeasts. Slonimski found that euflavine-treated cells of *S. cerevisiae* did not themselves become respiratory deficient but were inhibited in their production of certain of the respiratory enzymes and of the hereditary factors essential for transmission of respiratory competence to their buds. Since *S. cerevisiae* and related bakers' yeasts ferment strongly, either aerobically

or anaerobically, respiratory deficient buds are viable through the fermentative mechanism and continue to reproduce and form petite colonies. Both DeDeken (4) and Bulder (2) found that this agent blocked the synthesis of respiratory enzymes in the petite negative, as well as in the petite positive yeasts, and Bulder found, in fact, that petite negative yeasts were induced to produce microcolonies of respiratory-deficient cells capable of reproducing a few times but rarely developing viable petite colonies. He also found that some petite negative yeasts, although capable of fermenting glucose under reduced oxygen tension, would not grow anaerobically, and that the infrequent, viable petites obtained from the petite negative yeasts in general required some molecular oxygen for their growth.

Our cytological studies on representative groups of these yeasts thus indicate that the capacity for aerobic fermentation, glucose repression, and petite inducibility are related to, or are paralleled by, the mutability or stability of the mitochondria of the respective types.

Returning briefly to the intermittent electron-opaque membrane located in the peripheral region of the facultative anaerobes, this structure does not appear in our preparations of the strict aerobe, *R. gracilis*, and is less prominent in the weaker fermenting *S. fragilis* and *C. utilis* than it is in the strongly fermenting Carbondale *Saccharomyces*. Not having concentrated any cytochemical study on the membrane, we are not prepared to attribute to it any particular biochemical function. However, the structure seems of interest in view of the large number of papers associating the peripheral zone of the yeast cell with the locations of enzyme systems such as those of the Embden-Meyerhof-Parnas system of alcoholic fermentation (16) and various hydrolytic systems (3, 6–9, 14, 15, 19, 20).

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