RAPIDLY GROWING RESPIRATION-DEFICIENT VARIANT OF 
SACCHAROMYCES CEREVISIAE

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Since the description of the "petite colonie" of baker's yeast by Ephrussi et al. (Ann. Inst. Pasteur 76:351, 1949), many mutants of microorganisms with respiration deficiency have been studied. Some of these mutants have been proposed as screening organisms for the detection of antitumor antibiotics (Gause, Science 127:506, 1958). The impaired respiration of such mutants, however, satisfies only one of the properties of tumor cells. With respect to the rate of growth, the analogy breaks down, for cancer cells are characterized by rapid proliferation, while these mutants show a reduced growth rate. We attempted to isolate a mutant of a respiration-deficient (RD) variant of yeast which would grow more rapidly. It was felt that such a rapidly growing RD mutant might have better possibilities as a screening microorganism for the detection of antitumor substances than one which grew slowly.

The RD mutants used in this study were isolated from a culture of Saccharomyces cerevisiae haploid strain "yeast foam." The medium used for culture maintenance and plating was composed of 0.4% glucose, 0.4% yeast extract, 1% malt extract, and 2% agar. The same medium
with 5% glucose, but without agar, was used in all growth and inhibition studies. Davis and Mingioli (J. Bacteriol. 60:17, 1950) synthetic liquid medium supplemented with 0.4% sodium glutamate was used for inoculum preparation.

Inocula were prepared from cells of single colonies. Aerobic growth studies were carried out in a water-bath shaker at 35 C and anaerobic studies in Brewer jars. Comparative sensitivity studies of various agents were done by the serial dilution technique. Serially diluted tubes were read after 20 hr of incubation at 35 C. RD mutants were obtained by growing the wild-type yeast in a liquid medium containing 10 μg of acriflavine per ml for several successive transfers. The tetrazolium agar overlay technique of Ogur et al. (Science 125:928, 1957) and Nagai (Science 130:1188, 1959) was used to detect RD mutants. Respiration deficiency was confirmed by the glucose-limiting method of Ogur and St. John (J. Bacteriol. 72:500, 1956).

The acriflavine-treated yeast culture, when plated on acriflavine-free medium, gave rise only to RD colonies. However, the RD colony population was of two main types distinguishable by colony size. More than 99% of the colonies were uniformly small or “petite” in size, measuring 1 mm in diameter. The few remaining colonies were 2 mm in diameter and designated as large RD mutants. Wild-type colonies on control plates measured about 4 mm in diameter.

When platings were done of each of the RD types, the resulting clones were all respiration deficient, with more than 99% retaining their characteristic colonial size. A characteristic small colony “petite” and large colony “large RD” were selected for further study. Microscopic observations indicated no significant differences in cell size of the two RD mutants and the normal wild-type strain. The two RD types grew on glucose medium, but failed to develop on lactate and succinate media. Figure 1 shows the comparative growth rates of these yeast cultures under aerobic and anaerobic conditions. Under both conditions, the large RD mutant had a faster growth rate than the small RD mutant. Anaerobically, the large RD mutant grew even more rapidly than the parent wild type. When growth of the large RD mutant was compared with that of the small RD mutant in the presence of such antitumor substances as 6-diaz0-oxo-norleucine, amethopterin, and 5-fluorouracil, no differences in growth were indicated. In addition, the sensitivities of the wild-type yeast, the small RD mutant, and the large RD mutant to a variety of antitumor agents, respiratory enzyme inhibitors, and antibiotics were studied by serial dilution technique. There were no significant differences in sensitivities of these strains to the various substances.

**OXGALL MEDIUM FOR IDENTIFICATION OF PASTEURELLA PESTIS**

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The method proposed by us is based on the introduction of Oxgall (Difco) in the medium, and has been used with good results for the isolation and identification of *P. pestis* in spleen and liver homogenates of dead or moribund animals. The medium was prepared as follows. To distilled water were added Oxgall, 0.3%; agar (Difco), 1%;