An alternate respiratory pathway in *Candida albicans*

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Usual concentrations of antimycin A, rotenone and EDTA, individually or in combination, reduced aerobic growth rate and cell yield of *Candida albicans* to about half its normal level and to about the levels of previously-described acetate-negative, cytochrome-complete and aa₃-deficient variants which were little affected by the inhibitors. Anaerobic conditions (not affected by antimycin A) reduced growth rate and cell yield of all cultures – including that of a non-respiring aa₃,b-deficient mutant – to low, equal levels. Antimycin A but not rotenone prevented growth of the normal strain on ethanol medium. Cyanide and antimycin A blocked most of the respiration of the normal strain and cytochrome-complete variant, but did not affect that of the cytochrome aa₃-deficient mutant. Rotenone and EDTA did not affect respiration of any of the cultures. SHAM blocked cyanide- and antimycin A-insensitive respiration and prolonged the lag phases of the three respiring cultures, especially in the presence of antimycin A, but alone increased oxygen-uptake rate of the cytochrome-complete cultures while curtailting that of the cytochrome aa₃-deficient mutant. Resting cells, especially wild-type, grown in medium containing antimycin A exhibited lowered oxygen-uptake rate, which was increased upon the addition of cyanide or antimycin A. Antimycin A stimulated, but cyanide inhibited, respiration of cytochrome-complete cultures grown in the presence of rotenone but did not affect that of the cytochrome aa₃-deficient mutant. SHAM inhibited respiration of all antimycin A- or rotenone-grown cultures. The high rate of respiration of *C. albicans* in the presence of inhibitors for three sites of electron transport in the conventional oxidative pathway, the inhibition of this respiration by SHAM and its loss by the absence of cytochrome b, indicate an alternate oxidative pathway in this organism which crosses the conventional one at cytochrome b.

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INTRODUCTION

Previous work with *Candida albicans* and some of its respiratory mutants suggested an alternate, but secondary, respiratory pathway for this species (Kot et al., 1975). The present paper describes growth and respiratory studies with various electron transport inhibitors which provide more evidence for the alternate system.

MATERIALS AND METHODS

*Cultures. Candida albicans* ATCC 753 (B) and three of its acriflavine-induced, acetate-negative mutants: B-r14 with a complete cytochrome system, B-r86 deficient in cytochromes aa_3_, and B-r85 deficient in cytochromes aa_3_ and b, were grown and maintained on the glucose–yeast extract–peptone (YEP) medium previously described (Kot et al., 1975).

*Growth curves.* Growth curves were plotted from repeated turbidity readings of cultures in 250 ml Erlenmeyer side-arm flasks using 15 ml of synthetic medium similar to Bacto Yeast Nitrogen Base containing 1% glucose or ethanol as previously described (Kot et al., 1975). The effects of antimycin A, rotenone, ethylenediaminetetraacetic acid (EDTA) and salicyl-hydroxamic acid (SHAM) were determined by adding them to the medium, respectively, to final molar concentrations of 10^{-5}, 10^{-5}, 10^{-3} and 1.6 \times 10^{-3}. Antimycin A, rotenone and SHAM were dissolved in absolute ethanol; EDTA was dissolved in water.

Anaerobic growth curves were determined as above, except that the flasks were placed in a series of BBL anaerobic jars with the disposable GasPak hydrogen–carbon dioxide generators, catalysts and indicators all from the Baltimore Biological Laboratories, Baltimore, Maryland, such that a separate jar was used for each point of time on the growth curves.

*Respiration measurements.* Oxygen uptake was measured with a Model 53 Oxygen Polarimeter (Yellow Springs Instrument Co., Yellow Springs, Ohio). The cells were suspended at a concentration of about 9 \times 10^9 per ml in 0.05 M sodium phosphate buffer, pH 7.4. Glucose was used at a concentration of about 0.02 M, ethanol at about 0.36 M and inhibitors were added as indicated. The QO_2_ was calculated as ml of oxygen consumed per h per mg of dry yeast cells.

*Enzyme assays.* To seek a possible rationale for the failure of the cytochrome-complete B-r14 to grow on nonfermentable carbon sources, enzyme assays for its TCA cycle were compared with those of wild-type B. Cells grown for 24 h in glucose–YEP medium were harvested by centrifugation and washed 3 times with distilled water. The cells were re-suspended in 0.05 M phosphate buffer, pH 7.4, at 0–4°C at approximately 50% suspension by volume and broken with a Branson Sonifier (Branson Instruments, Inc., Stamford, Conn.) using a flat horn tip with a power setting of 7 tuned for maximum amperes. Intact cells were removed by centrifugation at 2000 \times g for 15 min at 4°C and assays were