Short Communication

Antimycin A-resistant Respiratory Pathway in *Ustilago maydis* and *Neurospora sitophila*¹

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Antimycin A inhibits electron transport at a site generally believed to be located between cytochromes b and c (10). However, certain respiratory systems such as those of *Rhodotorula glutinis* (8, 9) and *Symlocoparas foetidus* (skunk cabbage) (12) are insensitive to the antibiotic. Unestar and Gleason (13) showed that following an initial inhibition, antimycin A actually stimulates respiration of mycelia of a Saprolegnia sp. We have observed a similar phenomenon in conidia of *Neurospora sitophila* Shear and Dodge, treated with the antibiotic. Respiration of sporidia of *Ustilago maydis* (DeCandolle) Corda, on the other hand, is not inhibited initially by the antibiotic, but rather is immediately stimulated. A feature which distinguishes the respiration of antimycin A-treated cells of *N. sitophila* or *U. maydis* from that of untreated cells is the difference in sensitivity to oxygen tension. This report characterizes the antimycin A effects on respiration of these two fungi and also presents evidence that an alternate terminal oxidase operates in the cells after treatment with the antibiotic.

*N. sitophila* was grown at room temperature on agar medium (2) supplemented with 2 g/liter of yeast extract. Conidia from 4- to 7-day-old cultures were suspended in water or buffer solution (0.02 M phosphate containing 0.5 g/liter of MgSO₄·7H₂O, pH 6.4), filtered through cheesecloth, and washed twice with water or buffer. Sporidia of *U. maydis* (ATCC 14826) were grown in shake culture at 30 C in a liquid medium of the same nutrient composition used to grow *N. sitophila*. After 18 to 24 hr, sporidia were centrifuged from the medium and washed twice with buffer solution. Unless otherwise specified, respiratory measurements were made with cells suspended in a glucose-mineral salts solution (pH 6.4) supplemented with vitamins (2) but lacking a nitrogen source. In some experiments 0.25% (w/v) sodium acetate was substituted for glucose. Antimycin A was added in methanol. Final concentration of methanol in control and treated cultures did not exceed 1% (v/v). Cell suspensions were shaken at 25 or 30 C, and samples were withdrawn at appropriate intervals for measurement of oxygen consumption with a Gilson recording oxygen cathode (Oxygraph). Oxygen consumption under similar conditions was also measured manometrically. Levels of O₂ in the atmosphere different from that in air were obtained by flushing the vessels for 15 min with O₂/N₂ mixtures containing 1, 2, or 5% O₂. Glucose and antimycin A were added from the sidearms after the vessels were closed and equilibrated. The KCN-KOH procedure of Robbie (11) was used for cyanide treatments in manometric experiments.

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Oxygen uptake by conidia of *N. sitophila* utilizing glucose is inhibited about 75% after exposure for 30 min to 10 μg/ml of antimycin A. However, this inhibition declines, and by 3 hr the effect is one of respiratory stimulation. Respiration of sporidia of *U. maydis*, on the other hand, is immediately stimulated by the antibiotic (2-10 μg/ml). The stimulation persists for 5 hr or longer. A feature which distinguishes respiration of treated cells of either *U. maydis* or *N. sitophila* from that of untreated cells is a marked difference in sensitivity to low levels of oxygen. The rate of O₂ uptake by untreated sporidia of *U. maydis* is linear from solution saturation levels of O₂ (234 μM) to about 2% saturation. The rate of O₂ uptake by treated sporidia (utilizing glucose or acetate) begins to decline at about 20% saturation and declines quite rapidly at O₂ levels below 10% saturation (Figs. 1 and 2). Conidia of *N. sitophila* behave similarly, although a small decline in rate of O₂ uptake occurs in untreated conidia at O₂ levels above 2% saturation (Fig. 3).

The terminal oxidase of the rapid, cyanide-resistant respiration of *Arum spadix* tissue was reported to require a much higher O₂ concentration for saturation than the terminal oxidase of the slower, cyanide-sensitive respiration in other tissues of the same plant (5). However, Yocum and Hackett (14) concluded from their studies that at reduced O₂ tensions the diffusion rate of O₂ through the liquid-suspending medium, rather than a low affinity of the terminal oxidase for O₂, limits the respiration rate of aroid spadix tissue.

The following evidence indicates that a decrease in sensitivity of respiration of antimycin A-treated cells of *N. sitophila* and *U. maydis* to O₂ tension is not due to O₂ diffusion limitations. The rate of O₂ uptake of treated cells falls below that of untreated cells at O₂ levels around 5% saturation for *U. maydis* and at about 4% for *N. sitophila* and then declines much more rapidly in treated than in the untreated cells. The rate of consumption by untreated sporidia of *U. maydis* at 2.5% O₂ saturation is 3 or 4 times that of treated sporidia even though the rate of consumption by the latter sporidia at higher levels of O₂ is more than 1.5 times that of the former. When glucose was omitted from suspensions of *U. maydis*, a marked stimulation of endogenous respiration was produced by antimycin A; however, the maximal rate of O₂ uptake at high levels of O₂ in solution was only 65% of that of untreated sporidia oxidizing glucose. Nevertheless, sensitivity of the respiration to O₂ tension was comparable to that of antimycin A-treated cells oxidizing glucose.

Respiratory rates at various oxygen concentrations were determined from slopes of lines drawn tangent to points along curves made with the Oxygraph. Accuracy of the method was increased by expanding sensitivity of the instrument so that the normal chart span was used to record respiratory rates at oxygen levels between 25 and 0% saturation. The reciprocal of velocity...
was plotted against the reciprocal of O$_2$ concentration, and the O$_2$ concentration-supporting half-maximal respiratory rate (Km) was calculated. The Km-values for U. maydis sporidia treated with 2, 4, or 10 µg/ml of antimycin A ranged from 4.5 to 6% O$_2$ saturation, whereas those for untreated sporidia ranged from 0.5 to 0.6%. The values for treated and untreated conidia of N. sitophila were 5 and 0.6%, respectively. The O$_2$ molarity values at Km, which are presented in Table 1, show that both N. sitophila and U. maydis are nearly 10 times as sensitive to O$_2$ tension in the presence of antimycin A as in its absence.

The effect of O$_2$ level on respiration of N. sitophila and U. maydis was also determined manometrically. Results were consistent with those obtained in polarographic measurements. In an atmosphere of air at 25 C the Q$_{O_2}$ was 19 and 11, respectively, for treated (10 µg/ml) and untreated conidia of N. crassa. At an atmospheric level of 0.9% O$_2$, the Q$_{O_2}$ for treated conidia was 7.6, whereas at a level of 0.75% O$_2$, the Q$_{O_2}$ for untreated conidia was 10.5. The Q$_{O_2}$ in an atmosphere of air at 30 C was 143 and 79, respectively, for treated (2 µg/ml) and untreated sporidia of U. maydis. The Q$_{O_2}$ at approximately 1% O$_2$ in the atmosphere was 63 and 80, respectively, for treated and untreated sporidia.

Respiration of the two fungi was resistant to cyanide. The Q$_{O_2}$ of U. maydis sporidia was increased from 82 to 143 by 4.6 x 10$^{-4}$ M HCN. A concentration of 4.6 x 10$^{-4}$ M HCN produced a similar stimulation of O$_2$ uptake for about 1.5 hr, but at the end of 3 hr the rate was about equal to that of the control. Respiration of N. sitophila was inhibited about 50% after 1 hr by 4.6 x 10$^{-4}$ M cyanide, but after 3 hr there was little or no inhibition. No tests were made to determine whether the cyanide-resistant respiration was similar to antimycin A-resistant respiration in sensitivity to O$_2$ tension.

The respiratory stimulation and increased sensitivity to O$_2$ tension which follows antimycin A treatment is apparently the consequence of a shift in the pathway of electron transport to an alternate terminal oxidase. Appreciable evidence indicates that antimycin A specifically blocks electron transport between cytochromes b and c (1, 3, 6). Therefore, electrons are probably diverted to an alternate oxidase at a site preceding cytochrome c. Spectroscopic examination of U. maydis sporidia showed that cytochrome b remains reduced in the presence of antimycin A, whereas cytochromes c and a$_2$ are oxidized (4). The alternate pathway is apparently of considerable value to U. maydis for growth in the presence of the antibiotic because growth of a mutant lacking the system is much more sensitive to antimycin A than that of the wild type (4). The antimycin A-resistant respira-
Stimulation of respiration in U. maydis by cyanide probably results from a shift in pathway of electron transport which by-passes phosphorylation sites rather than from a typical uncoupling such as that produced by 2,4-dinitrophenol. It seems likely, at least in U. maydis, that the same alternate electron transport pathway is utilized when either cyanide or antimycin A is present. Such is reported to be the case in skunk cabbage mitochondria (12).

LITERATURE CITED