EFFECT OF OXYGEN AND CARBON DIOXIDE ON THE MITOCHONDRIA OF *SCLEROTIUM ROLFSII**

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The number of mitochondria apparently vary with the energy requirements of the cell (see Rouiller 1960). Although swelling and multiplication of mitochondria have been observed to occur in cells, a quantitative analysis of these changes has not been recorded. Matile and Bahr (1968) have recently provided electron microscopic evidence of the heterogeneity of density, mass, and volume of the mitochondrial population in respiring baker's yeast. There are reports in the literature of the complete absence of mitochondria in yeast cells growing under anaerobic conditions (see Marchant and Smith 1968). Griffin and Nair (1968) demonstrated that the growth of *Sclerotium rolfsii* was inhibited by concentrations of oxygen below 4% and by concentrations of carbon dioxide above 0·03%. It was, therefore, thought worthwhile to study the changes in mitochondria of this fungus when the cells are subjected to external stresses of low oxygen and high carbon dioxide levels.

Methods

Cultures of *S. rolfsii* maintained at 25°C on malt–vegemite medium (Nair *et al.* 1969) were used. The diffusion-column technique of Griffin *et al.* (1967) was adopted to grow the fungus under different concentrations of oxygen and carbon dioxide. The following tabulation gives the compositions of the four gas mixtures used and the treatment numbers assigned to them (partial pressures are approximate values only):

Treatment	Concentration (%) of:			Partial Pressure (mmHg) of:		
	N_2	O_2	CO_2	N_2	O_2	CO_2
1	78·1	20·9	0·03	594·0	158·0	0·22
2	80·0	1·0	0·03	608·0	7·6	0·22
3	80·0	0·0	20·0	608·0	0·0	152·0
4	80·0	10·0	10·0	608·0	76·0	76·0

The fungus was grown for 8 days on the diffusion column at the different concentrations of oxygen and carbon dioxide. Mycelia were then fixed in 6·5% glutaraldehyde in cacodylate buffer at pH 7·6 for 4 hr at 4°C followed by post-fixation in 1% osmium tetroxide in veronal acetate buffer at pH 7·6 for 2 hr at 4°C. The methods used in the preparation of fungal hyphae for electron microscopy were the same as described earlier (Nair *et al.* 1969). Cross and longitudinal sections of mitochondria were taken into consideration in determining the effect of oxygen and carbon dioxide. Areas of mitochondria were cut out (mitochondrial profiles) from outline drawings made from electron micrographs of the fungal cells, and weighed; the values so obtained were taken as the weight of mitochondria. The cell volumes were determined by assigning rectangular shape

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for longitudinal sections of hyphal cells and circular shape for cross sections of hyphae. Results are expressed as weight of mitochondria per volume of cell and weight of mitochondria as percentage of cell volume (Table 1). Ten replicates each of cross and longitudinal sections of hyphal cells were included in the analysis of the data. Regression analysis was done on the weight over the number of mitochondria per cell (Fig. 1).

TABLE 1

EFFECT OF CARBON DIOXIDE AND OXYGEN ON THE MORPHOLOGY OF MITOCHONDRIA IN *S. ROLFSII*

Values in parenthesis denote dimensions of mitochondria that are less frequent

Treatment	Dimensions of Mitochondria (μ)	Weight of Mitochondria per Volume of Cell (g)	Weight of Mitochondria as % of Cell Volume
	Data based on cross sections of mitochondria		
1	0·4–0·7(1·2) by 0·3–0·5(1·0)	0·0003	0·03
2	(0·2)0·4–0·6 by (0·08)0·2–0·6	0·00004	0·004
3	(0·3)0·6–0·8 by (0·2)0·5–0·51	0·00001	0·001
4	(0·2)0·4–0·5 by (0·1)0·3–0·5	0·00002	0·002
	Data based on longitudinal sections of mitochondria		
1	0·6–0·8(1·5) by 0·4–0·5(1·0)	0·0009	0·09
2	(0·4)0·8–1·1 by (0·05)0·1–0·12	0·0004	0·04
3	(0·4)1·0–1·3 by (0·08)0·2–0·3	0·0005	0·05
4	(1·0)2·8–6·6 by (0·01)0·02–0·3	0·0002	0·02

Results and Discussion

Mitochondria multiplied more than threefold in cells grown under low oxygen (1%) and high carbon dioxide (20%) conditions; however, their average weight or weight per volume of cell was much less when compared with those in cells grown in concentrations of oxygen and carbon dioxide normally present in air. Results of regression analysis of weight over the number of mitochondria per cell are given in Figure 1. Cultures of *S. rolfsii* growing at 1% oxygen, 20% carbon dioxide, and 10 : 10% oxygen–carbon dioxide were transferred back to air, and the fungal cells were examined after growth for 48 hr. It was observed that all these cells had normal type of mitochondria.

Quantitative analysis of the morphological changes in mitochondria of cells subjected to external stresses such as low oxygen and high carbon dioxide concentrations support earlier observations of a qualitative nature. Based on results obtained in the present work, it appears that, although the growth of *S. rolfsii* is inhibited by low oxygen and high carbon dioxide concentrations (Griffin and Nair 1968), the fungus responds to such external stresses at the subcellular level by increasing the number or concentration of mitochondria within the cell. This suggests an increased requirement for ATP. Nair *et al.* (1969) have shown that respiratory enzymes are located in the mitochondria of *S. rolfsii* and that there is movement of these organelles between cells of the hyphae. Multiplication of mitochondria in cells growing under conditions of low oxygen and high carbon dioxide might facilitate

the respiratory processes of these cells. The ability of *S. rolfsii* to resynthesize the normal type of mitochondria on transfer to aerobic conditions indicate that this fungus still retained the ability to carry out normal mitochondrial organization. This behaviour closely resembles that observed in yeast by Osumi and Katoh (1967).

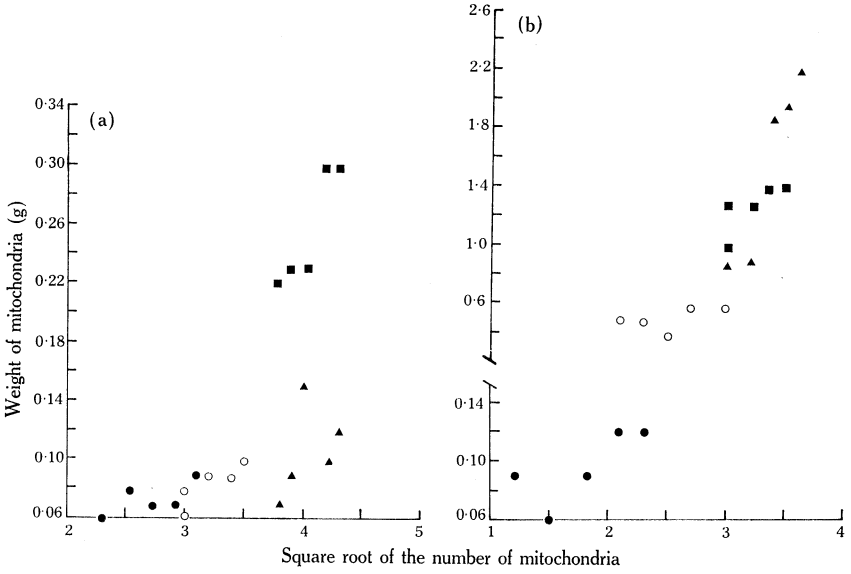

Fig. 1.—Results of regression analysis of weight over the number of mitochondria per cell at different concentrations of oxygen and carbon dioxide, based on data from cross sections (*a*) and longitudinal sections (*b*) of mitochondria. ● Treatment 1; ■ treatment 2; ▲ treatment 3; ○ treatment 4.

S. rolfsii usually occurs in well-aerated soils. Reduction in germination and growth of sclerotia in non-aerated soils is considered to be an effect of accumulation of carbon dioxide. Under such conditions the fungus would remain dormant. The ability of the fungus to recover its normal process of mitochondrial organization on transfer from anaerobic to aerobic conditions would facilitate effective colonization of host tissue. This appears to be an important aspect of its ecological behaviour in soil.

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