THE ULTRASTRUCTURE OF MITOCHONDRIA IN *BLASTOCLADIA PRINGSHEIMII* REINSCH

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Abstract

Blastocladia pringsheimii Reinsch is a zoosporic fungus with a strongly fermentative mode of metabolism. Mitochondria were observed by the electron microscope in thin sections of cells grown aerobically on either glycerol or glucose as carbon sources. The ultrastructure of the cristae was similar to that previously observed in *Blastocladia ramose* Thaxter, which is also strongly fermentative but morphologically different. The possible evolutionary relationship of the genus *Blastocladia* to other members of the Chytridiomycetes is discussed. This discussion includes both obligately aerobic and obligately anaerobic species.

F.H. Gleason *et al.* (2002). The ultrastructure of mitochondria in *Blastocladia pringsheimii* Reinsch. *Australasian Mycologist* 21 (2): 41–44.

Introduction

The genus *Blastocladia* belongs to the Chytridiomycetes, a large class of aquatic fungi, which in general is considered to be obligately aerobic (Sparrow 1960). The known exceptions are the genus *Blastocladia* (Order Blastocladiales), which includes at least two facultative anaerobes, and five genera of rumen chytrids (Order Neocallimasticales), which are obligate anaerobes (Gleason & Gordon 1989, Held *et al.* 1969, Trinci *et al.* 1994). Sparrow (1960) describes ten species of *Blastocladia* based upon significant differences in the morphology of the sporangia and the basal cells. These fungi are commonly found growing on decaying twigs and fruits in freshwater habitats usually with a reduced concentration of dissolved oxygen (Sparrow 1960). Physiological studies have been conducted with only two of the ten species, *B. pringsheimii* and *B. ramosa*.

Blastocladia pringsheimii Reinsch was first described by Cantino (1949) as a zoosporic fungus with a strongly fermentative type of metabolism. The molar growth yield of cells produced (mg dry weight/mole of glucose consumed) and the production of acid by-products on a medium containing glucose was influenced only slightly, if at all, by the concentration of oxygen in the medium (Cantino 1949, Held *et al.* 1969). A second morphologically different species of this genus, *Blastocladia ramosa* Thaxter, was also found to be strongly fermentative (Craseman 1957). Held *et al.* (1969) provided further evidence for a strongly fermentative type of metabolism in both *B. pringsheimii* and *B. ramosa* by different methods. In particular they demonstrated a capacity for oxygen uptake which was much smaller than for related aerobic fungi. In addition they discovered sparsely distributed organelles, which were bound by a double unit membrane but without clear development of cristae, in both species of *Blastocladia*, but only a micrograph of *B. ramosa* was included in the paper. This raised the possibility that the two *Blastocladia* species either have defective mitochondria or no mitochondria at all. Using greatly improved methods of fixation, Lingle & Barstow (1983) observed many cristate mitochondrial profiles in zoospores of *B. ramosa* but with substantially fewer cristae than in the related aerobic fungi in the Order Blastocladiales. This might indicate a lower capacity for aerobic respiration in *B. ramosa*. However, Lingle & Barstow (1983) did not investigate the ultrastructure of zoospores of *B. pringsheimii*, and there is no information in the literature on the presence of christate mitochondria in any stage of the life cycle in *B. pringsheimii*. In addition to morphological differences between the two species, the sources of carbon used and the acid fermentation products produced during the fermentation by the two species of *Blastocladia* are different (Gleason & Gordon 1989).

The purpose of our study was to document the presence of cristate mitochondria in cells of *B. pringsheimii* which were grown on a nonfermentable substrate in order to stimulate an aerobic type of respiration. Then we wished to compare the ultrastructure of mitochondria in this species with those in *B. ramosa* and with obligately

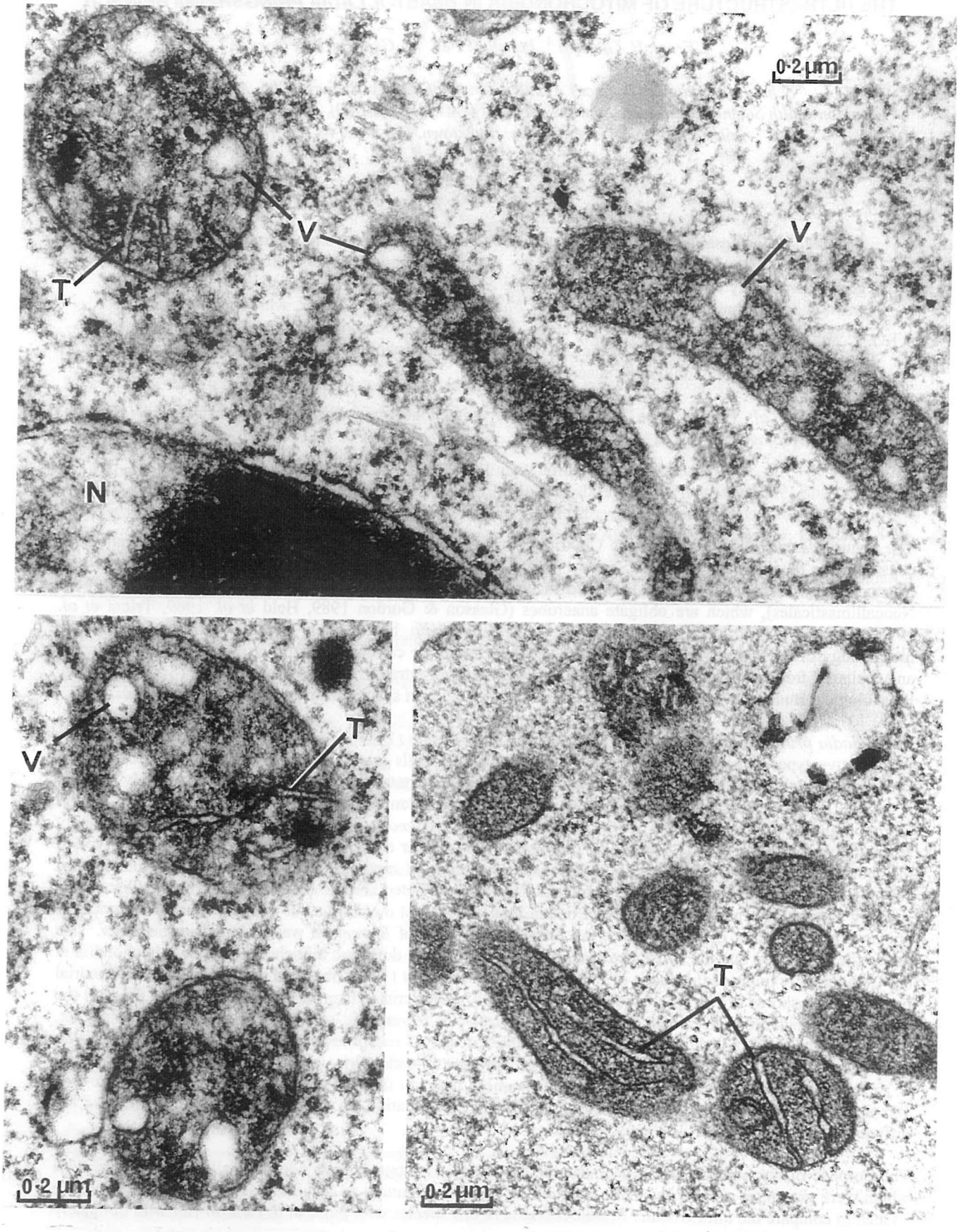

Figure 1. Mitochondria in cells of *Blastocladia pringsheimii* Reinsch grown aerobically in a medium containing glycerol as a carbon source. Cristae appear either vesicular (V) or tubular (T) in profile in section. Part of the nucleus (N) is also visible. Portions of sections through three different cells were included. Scale bar equals 0.2 micrometer.

aerobic members of the Chytridiomycetes previously described in the literature, and finally to discuss the evolutionary significance of the capacity for fermentation and respiration in the genus *Blastocladia*.

Materials and Methods

In order to stimulate an aerobic type of metabolism we chose an isolate of *Blastocladia pringsheimii* Reinsch (CR62) which will grow on a medium containing glycerol, a non fermentable substrate, as a carbon source in the presence of oxygen but not in the absence of oxygen (Gleason & Gordon 1989). The isolate of *B. ramosa* used by Lingle & Barstow (1983) (54-14) cannot utilise glycerol as a carbon source (Craseman 1957, Gleason & Gordon 1989). *Blastocladia pringsheimii* CR62 was obtained from the culture collection at the Department of Botany, University of California (Berkeley, Calif., U.S.A.). The growth media contained either glycerol or glucose 3.0 g, yeast extract (Difco) 1.25 g, bacto peptone (Difco) 1.25 g, KH_2PO_4 1.36 g, Na_2HPO_4 0.71 g, $MgSO_4.7H_2O$ 0.12 g and bromo cresol purple (for pH adjustment) 1 mg per litre. Volumes of media were dispensed (25 ml) into 100 ml serum bottles with air in the gas phase then capped with butyl rubber stoppers and aluminium crimp seals (Bellco Glass, Inc., Vineland, New Jersey, U.S.A.). When necessary during growth, the pH of the media was adjusted to the original value of 6.7 by addition of 0.5M NaOH dropwise with a syringe, while observing the colour of the medium. The cultures were grown for six days on glycerol and three days on glucose at room temperature.

A solution of 6% glutaraldehyde in 0.2M sodium cacodylate buffer (pH 7.2) was then added to an equal volume of fungal cells suspended in the culture medium to give a final concentration of 3% glutaraldehyde, and the fungi were fixed for two hours at room temperature. After washing three times with 0.1M sodium cacodylate buffer, the cells were post fixed for two hours with 1% osmium tetroxide in 0.1M sodium cacodylate buffer at room temperature. The cells were washed again three times with buffer, suspended in 3% agarose, dehydrated through a graded series of ethanol and ethanol/propylene oxide solutions, and then infiltrated and embedded in araldite (CY212, Agar Aids). Ultrathin sections were cut using a diamond knife in a LKB Ultratome IV ultramicrotome. The sections were stained using stabilised solutions of uranyl acetate and lead citrate in an LKB 2168 Ultrostainer (Carlsberg System) and photographed using a Jeol JEM-100S electron microscope.

Results and Discussion

Mitochondrial profiles were observed by the electron microscope in thin sections of cells of *B. pringsheimii* grown aerobically in media containing either glycerol or glucose. All parts of the fungus appeared to contain mitochondria, *i.e.* sporangia, zoospores, basal cells and rhizoids. There appeared to be more mitochondria in cells grown in glycerol than in cells grown in glucose, but quantitative measurements were not attempted. Thin sections of mitochondria in three cells grown in glycerol are shown in Figure 1. The cristae appeared either vesicular or tubular in profile in the sections. Some had one type of profile, some had both types and some had very little invagination of the inner membrane. This was probably due to the angle of the section through the mitochondria and differences in the extent of development of cristae in individual mitochondria. However, we did not attempt to examine the three dimensional shape of the cristae with serial sections, but plate-like cristae are commonly found in Chytridiomycetes.

The ultrastructure of the cristae in mitochondria observed in all parts of *B. pringsheimii* grown aerobically either on glucose or on glycerol is similar to that previously described by Lingle & Barstow (1983) in zoospores from *B. ramosa* grown aerobically on glucose. It is also similar to that in other strongly aerobic members of the Order Blastocladiales described by Lingle & Barstow (1983).

Blastocladia pringsheimii produced acid by-products from growth on both glucose and glycerol as carbon sources although the total concentration of acid produced was less on the glycerol medium (Cantino 1949, Gleason & Gordon 1989). Oxygen is not required for the fermentation of glucose to lactic acid but is required for the catabolism of glycerol, a nonfermentable substrate (Gleason & Gordon 1989). Mitochondria presumably play a minor role, if any, in metabolism during the fermentation of glucose to lactic acid, especially under the reduced oxygen concentrations often found in the normal habitat, but play a major role in the oxidation of glycerol to glyceraldehyde before it can enter the glycolytic pathway.

Since the ultrastructure of the mitochondria in both species of *Blastocladia* is similar, ultrastructural differences cannot account for the differences in ability to use glycerol and the differences in the type of acid fermentation products in the two species. However, the presence of cristate mitochondria in both species indicates some capacity for aerobic respiration, although it is possible that they may be in the process of losing some metabolic pathways normally used in aerobic respiration. The precise nature of the roles of mitochondria in these fungi remains to be determined by isolation of these organelles and examination of the enzymatic systems present within them, especially the membrane bound enzymes found in the cristae.

Without molecular data the evolutionary relationship of these two species of *Blastocladia* with other members of the Blastoladiales remains unknown. The genus *Blastocladia* has adapted to freshwater habitats usually with a reduced concentration of dissolved oxygen (Sparrow 1960). It is also possible that *Blastocladia* can grow anaerobically when oxygen is removed as a result of respiration in other micro-organisms. However, the oxygen concentration range in aquatic habitats where *Blastocladia* has been observed growing has never been accurately measured and reported in the literature. Nonetheless, the two *Blastocladia* species are the only Chydridiomycetes that can be grown both aerobically and anaerobically in the laboratory, and thus are considered to be facultatively anaerobic. Most other fungi in the Chytridiomycetes are obligately aerobic.

It is interesting to note that obligately anaerobic rumen chytrids, which have adapted to the anaerobic microenvironment found in parts of the rumen or hindgut of mammals, do not contain any mitochondria but instead contain hydrogenosomes and produce fermentation products not found in *Blastocladia* (Trinci *et al.* 1994). Presumably the rumen chytrids either have lost the capacity to synthesize mitochondria or have evolved from protistan cells that never had mitochondria. No hydrogenosomes were seen in *B. pringsheimii* under the growth conditions used in the present study.

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