

and *TaqI* differentiated the three ruminal strain from the seven equine strains. In consequence five groups of strains were differentiated: group I include A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub> and P<sub>2</sub>, group II: A<sub>5</sub>, group III: P<sub>3</sub> and P<sub>4</sub>, group IV: V<sub>1</sub> and V<sub>2</sub>, group V: M<sub>1</sub>.

Thus, the ITS analysis allowed differentiation of the strains according to their origin.

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**Attachment to cellulose and subsequent development of the anaerobic rumen fungus *Neocallimastix frontalis* strain RE1.** AJ Richardson<sup>1</sup>, GW Gooday<sup>2</sup>, CS Stewart<sup>1</sup> (<sup>1</sup>Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK; <sup>2</sup>Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Forsterhill, Aberdeen, AB25 2ZD, UK)

Anaerobic fungi colonize a wide range of plant materials present in ruminant diets including maize, lucerne, straw and grasses. Degradation of cellulosic substrates by anaerobic fungi in the rumen involves the attachment and encystment of zoospores on plant surfaces and their subsequent maturation into zoosporangia with rhizoidal systems which penetrate and degrade the plant material. Relatively little is known about the mechanism of adhesion by rumen fungi. Radiolabelling has been used to quantify the effects of plant secondary metabolites on attachment of anaerobic fungal biomass to cellulose [1]. Using a similar technique we have quantified the attachment to cellulose of separated, <sup>14</sup>C-labelled zoospores and zoosporangia of the anaerobic rumen fungus

*Neocallimastix frontalis* strain RE1 Both zoospores and zoosporangia were found to make a contribution to the colonization process *in vitro*.

Anaerobic fungi belong to the class Chytridiomycetes, order Spizellomycetales. In the Blastocladiales, an aerobic order of the Chytridiomycetes, almost all of the genera survive adverse conditions by producing resistant sporangia with cell walls which are frequently thickened and melanized [2]. The isolation of anaerobic fungi from samples of fresh and air-dried faeces and saliva from various herbivores has led to the suggestion that an aero-tolerant resistant stage similar to the resistant structures produced in aerobic chytrids may be responsible for the survival of these fungi outside the rumen. The formation of pigmented, melanized resistant structures in a *Neocallimastix* sp. has been reported [3] and these were considered to be the aero-tolerant resistant stage. When *N. frontalis* strain RE1 was grown on filter paper dark brown pigmented zoosporangia appeared after 4-5 d but these differed in some respects from those previously described in both aerobic and anaerobic chytrids. The cell walls of the zoosporangia were not thickened and the pigment was present in the cytoplasm which contracted into a compact mass within the cell. Histochemical tests showed that the pigment was not melanin. Furthermore pigmented zoosporangia developed when strain RE1 was incubated in the presence of tricyclazole, PP389 or glyphosate, compounds which block melanin biosynthesis in fungi. Nonetheless, these structures may represent a resting stage in the life-cycle of strain RE1.

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### **A common evolutionary origin for mitochondria and hydrogenosomes.**

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Although it has been known for about ten years that anaerobic fungi contain hydrogenosomes [1] little biochemical or morphological data is available about these organelles compared to their homologues in *Trichomonas vaginalis*. This paucity of data makes it difficult to determine the evolutionary origin of these organelles.

Hydrogenosomes are a site of substrate-level phosphorylation in anaerobic fungi and therefore of great energetic importance for these amitochondriate eukaryotes. For this reason and because of ultrastructural similarities it has been suggested that hydrogenosomes are derived from mitochondria [2,3]. The presence of the typical anaerobic prokaryotic enzymes hydrogenase and pyruvate:ferredoxin oxidoreductase in hydrogenosomes has inspired Müller [4] to suggest that hydrogenosomes originated by the endosymbiotic uptake of an anaerobic Gram-positive bacterium. A third hypothesis regards peroxisomes as the progenitor organelle for hydrogenosomes [5,6].

Our recent work has provided evidence that fungal hydrogenosomes are probably derived from mitochondria. This data includes primary sequences of hydrogeno-

somal enzymes which seem to have mitochondrial-like targeting signals [3,7]. Recently we discovered that, like mitochondria, fungal hydrogenosomes play a role in  $Ca^{2+}$ -homeostasis [8] and that fungal hydrogenosomes are surrounded by a double membrane like all other hydrogenosomes [9]. In summary, all these data suggest a common evolutionary origin for mitochondria and hydrogenosomes.

Professor Rudolf A. Prins passed away on the 26<sup>th</sup> February 1997. For many years he has been an inspiration to those in the field of fungal hydrogenosomes.

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