Distribution of anaerobic fungi in the digestive tract of cattle and their survival in faeces

DAVID R. DAVIES,^{1,2} MICHAEL K. THEODOROU,^{1*} MICHELLE I. G. LAWRENCE^{1,2} and ANTHONY P. J. TRINCI²

¹AFRC Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, UK

²Microbiology Group, Department of Cell and Structural Biology, School of Biological Sciences, Stopford Building, University of Manchester, Manchester M13 9PT, UK

(Received 22 October 1992; revised 8 February 1993; accepted 10 February 1993)

A most probable numbers procedure was used to enumerate populations of anaerobic fungi in the digesta and faeces of cattle. Anaerobic fungi were isolated from the rumen, omasum, abomasum, small intestine, caecum, large intestine and faeces. By determining the amount of digesta in each organ of the digestive tract, it was possible to estimate the total population of anaerobic fungi in cattle and make comparisons between populations in different organs. In addition to enumerating anaerobic fungi in freshly collected samples, they were quantified in digesta and faeces which had been dried at ambient temperature and stored in air for up to 9 months. These experiments showed that a higher proportion of the anaerobic fungi present in the hindgut and faeces were able to withstand desiccation than those present within the gastric and pre-gastric organs. Our results support the hypothesis that the life cycle of anaerobic fungi consists of three stages; the motile zoospore, the vegetative thallus and an aero-tolerant survival stage (cyst or resistant zoosporangium).

Introduction

The digestive tract of many herbivores has evolved such that the plant biomass ingested can be degraded in a way which best serves the nutrition of the animal. In this respect, ruminants are particularly dependent on the activity of micro-organisms and it is now accepted that their gut microflora consist of a diverse population of bacteria, protozoa and fungi which can grow under highly anaerobic conditions.

Anaerobic fungi were first isolated from ruminants (cattle and sheep) by Orpin (1975) and Bauchop (1979) and have since been found in the digesta and faeces of numerous other ruminant and monogastric herbivores, including post-gastric fermenters such as the elephant, horse and rhino (Theodorou *et al.*, 1992). Lowe *et al.* (1987) were the first to isolate anaerobic fungi from the faeces of sheep. These authors obtained isolates from faeces which were morphologically similar to *Neocallimastix* and *Caecomyces* species found in the rumen.

In further studies, Milne *et al.* (1989) isolated anaerobic fungi from the faeces of a wide range of herbivores and demonstrated the resistance of these organisms to desiccation by isolating them from faeces that had been dried and stored in air for up to 18 weeks. More recently, Theodorou *et al.* (1990) developed a most probable numbers (MPN) procedure for the enumeration of anaerobic fungi in rumen digesta and faeces. Using this technique, it was shown that ruminants could contain approximately equivalent numbers of anaerobic fungi (relative to digesta or faecal dry matter) in fresh faeces as in their rumen digesta. Unlike anaerobic fungi in faeces, anaerobic fungi in rumen digesta fail to survive drying (Milne *et al.*, 1989; Theodorou *et al.*, 1990).

Representatives of four of the recognized genera of anaerobic fungi, *Neocallimastix*, *Piromyces*, *Caecomyces* and *Orpinomyces*, commonly found in rumen digesta, have been obtained from both fresh and air-dried faeces (Lowe *et al.*, 1987; Milne *et al.*, 1989; Wubah *et al.*, 1991a; D. R. Davies, M. K. Theodorou, A. P. J. Trinci & B. B. Nielsen, unpublished results). Where anaerobic fungi have been found in faeces, particularly in the faeces of wild herbivores, their presence in the digestive tract is usually assumed. Although anaerobic fungi in the rumen have been of considerable interest since their discovery,

* Author for correspondence. Tel. (0970) 828255; fax (0970) 828357.

Abbreviations: t.f.u., thallus forming units; MPN, most probable number; DM, dry matter.

the ability of these obligately fermentative organisms to survive in other organs of the digestive tract and in faeces has been given relatively little attention.

One hypothesis to explain the large populations of resistant anaerobic fungi found in faeces is that they can produce a resting stage (a survival structure) in their life cycle (Theodorou *et al.*, 1990). In the work reported here, fresh and dried samples of faeces and digesta from each organ of the digestive tract of cattle were investigated for the presence of anaerobic fungi. The results obtained provide the first quantification of anaerobic fungi in the entire ruminant digestive tract and are consistent with the hypothesis that anaerobic fungi are able to produce resistant structures.

Methods

Animals sampled. Entire digestive tract studies, in which anaerobic fungi were enumerated in faeces and in digesta from each of the digestive tract organs, were performed on three groups of animals, each consisting of three animals as follows: (a) lactating Friesian/Holstein dairy cows, where enumerations were performed on fresh digesta and faeces; (b) 8-month-old Friesian/Holstein steers, where enumerations were performed on digesta and faeces prior to and after drying and storage in air for 7 d; and (c) 20-month-old Friesian/Holstein steers, where enumerations were performed on digesta and faeces prior to and after drying and storage in air for up to 9 months. All animals were fed an *ad libitum* perennial ryegrass (*Lolium perenne* L.) silage diet with supplements. Dairy cows received per day 3 kg barley-based concentrates containing 20% (w/w) crude protein. Fishmeal was added to the diet of the 8-month-old steers at a rate of 10% (w/w) dry matter (DM) of the daily silage intake. The diet of the 20-month-old steers was not supplemented. Water and mineral blocks were freely available to all animals at all times.

Sampling procedure. The alimentary tract was removed intact from the carcass immediately after slaughter and dissected into organs, after tying off the various sections with bailer twine to reduce the likelihood of cross-contamination between organs (particularly between the abomasum and small intestine). Digesta samples (approximately 200–400 g fresh wt) for fungal enumeration, DM and pH determinations were removed from the rumen, omasum, abomasum, small intestine, caecum and large intestine; an approximately equivalent amount of faeces was collected from animals prior to slaughter. The pH of digesta and faeces was determined using a mobile pH meter (Philips). Depending on the part of the tract being sampled, the total gut contents from each organ was determined by one of two methods. Either the contents of the entire organ were emptied into a container and weighed directly, or the organ and its contents were weighed, the contents removed and the empty organ weighed after washing. This latter procedure was used for the omasum where it was impractical to remove all of the digesta contents without washing. The time taken between slaughter and fungal enumeration was approximately 1 h and during this period samples were maintained at about 37 °C.

Enumeration of anaerobic fungi. Anaerobic fungi were enumerated as thallus forming units (t.f.u.) per g digesta or faecal DM, and per organ, using wheat straw as the substrate [1% (w/v) wheat straw ground through a 1 mm dry mesh screen], as described by Theodorou *et al.* (1990). Enumerations were performed on 5 g (fresh wt) quantities of fresh sample or samples which had been dried and kept in air at ambient temperature for up to 9 months. The positive and negative

tubes from MPN enumerations, using three replicates per dilution and 10-fold dilutions, were quantified according to the method of Ross (1987).

The enumeration medium, designated Medium C, was based on that of Orpin (1977). Medium C contained (l⁻¹): salts solution I (150 ml), salts solution II (150 ml), centrifuged (clarified) rumen fluid (150 ml), Yeast extract (Oxoid) (2.5 g), Trypticase peptone (Becton and Dickinson) (10.0 g), NaHCO₃ (6.0 g), resazurin solution (0.1%, w/v) (1.0 ml), L-cysteine hydrochloride (1.0 g). Salts solution I contained K₂HPO₄ (3.0 g l⁻¹) in distilled water. Salts solution II contained each of the following components (g l⁻¹) dissolved in distilled water in the following order: KH₂PO₄ (3.0), (NH₄)₂SO₄ (6.0), NaCl (6.0), MgSO₄ · 7H₂O (0.6), CaCl₂ · 2H₂O (0.6).

With the exception of L-cysteine hydrochloride, each component was added in the amount indicated and the medium made up to 1 l with distilled water. The L-cysteine hydrochloride was added after the medium was gassed for 1 h with oxygen-free CO₂. Aliquots (45 ml) were dispensed into 60 ml serum bottles using anaerobic techniques and autoclaved at 121 °C for 15 min. Prior to use in the MPN procedure, 1 ml of chloramphenicol solution was added to each bottle of Medium C. The antibiotic solution contained 5 mg chloramphenicol ml⁻¹ dissolved in 50% (v/v) aqueous ethanol.

Results

pH and dry matter of digesta and faeces

The pH of digesta in pre-gastric (reticulo-rumen and omasum) and post-gastric (caecum and large intestines) organs and the small intestines, and of faeces of all animals, ranged from neutral to slightly acidic (Table 1). In the abomasum, which is the site of gastric digestion, digesta contents were more acidic, with mean pH values ranging from 4.9 for dairy cows to 3.3 for both growing and mature steers (Table 1).

Within each group of animals, the total amount of digesta DM was highest in the rumen, ranging from 3881 g in growing steers to 9656 g in lactating dairy cows (Table 1). Other organs contained much less DM, ranging from 151 g in the large intestine of growing steers to 1077 g in the small intestine of dairy cows (Table 1). Distribution of DM throughout the digestive tract was remarkably consistent across all animals with approximately 84.0% contained within the pre-gastric organs, approximately 10.6% in the abomasum and small intestines and only about 5.4% in the post-gastric organs. Values for total digesta DM content were used subsequently, together with data obtained from the MPN procedure, to calculate fungal t.f.u. per organ and fungal t.f.u. per g digesta or faecal DM.

Fungal populations in digesta and faeces

Fungal populations (enumerated as t.f.u. per g DM and t.f.u. per organ) in digesta and faeces from dairy cows, growing steers and mature steers are shown in Table 2. Except for the small intestine, where anaerobic fungi were cultured from two out of three dairy cows and two

Table 1. *pH and dry matter content in each organ of the digestive tract and in faeces of cattle*

Each value represents the mean of three observations $\pm$ SD. The animals were: (a) Friesian/Holstein dairy cows of mean live weight 570 kg and fed *ad libitum* silage diet with 3 kg barley-based concentrates per d; (b) 8-month-old Friesian/Holstein steers of mean live weight 244 kg and fed an *ad libitum* silage diet, with fishmeal added at a rate of 10% dry matter of daily silage intake; (c) 20-month-old Friesian/Holstein steers of mean live weight 497 kg and fed an *ad libitum* silage diet without supplements. Total dry matter (DM) was determined by collecting the entire digesta from each organ, obtaining a fresh matter weight and then adjusting for the percentage dry matter of representative samples.

Organ	Dairy cow		Growing steer		Mature steer	
	pH	Total DM per organ (g)	pH	Total DM per organ (g)	pH	Total DM per organ (g)
Reticulo-rumen	5.7 $\pm$ 0.64	9656 $\pm$ 1085	6.7 $\pm$ 0.13	3881 $\pm$ 584	6.5 $\pm$ 0.21	6339 $\pm$ 476
Omasum	6.3 $\pm$ 0.32	463 $\pm$ 244	6.6 $\pm$ 0.17	329 $\pm$ 97	6.6 $\pm$ 0.12	1054 $\pm$ 143
Abomasum	4.9 $\pm$ 0.44	290 $\pm$ 261	3.3 $\pm$ 0.47	152 $\pm$ 10	3.3 $\pm$ 0.10	252 $\pm$ 27
Small intestine	7.0 $\pm$ 0.34	1077 $\pm$ 367	5.7 $\pm$ 0.04	443 $\pm$ 66	6.7 $\pm$ 0.00	500 $\pm$ 39
Caecum	6.7 $\pm$ 0.06	269 $\pm$ 114	7.0 $\pm$ 0.12	169 $\pm$ 5	6.7 $\pm$ 0.06	179 $\pm$ 70
Large intestine	6.6 $\pm$ 0.09	290 $\pm$ 85	6.9 $\pm$ 0.05	151 $\pm$ 32	6.7 $\pm$ 0.00	277 $\pm$ 69
Faeces	6.5 $\pm$ 0.17	NA	6.9 $\pm$ 0.07	NA	6.7 $\pm$ 0.00	NA

NA, Not applicable.

Table 2. *Fungal t.f.u. per g dry matter (DM) and t.f.u. per organ for digesta and faeces from cattle*

Each value represents the mean of three observations. The MPN enumerations used 10-fold dilutions and three replicates per dilution. Total t.f.u. per organ were calculated from the total digesta dry matter per organ (see Table 1) and the t.f.u. per g DM for that organ. The lower 95% confidence limits for the enumerations were approximately 4.0–5.0 fold lower than the values presented in the Table. With the exception of the caecum in growing and mature steers where the upper 95% confidence limits were 5.4- and 6.2-fold higher than the values presented in the Table, the upper limits were approximately 3.0–4.0-fold higher than the values presented. NA, Not applicable.

Organ	t.f.u. (g DM) ⁻¹			t.f.u. per organ		
	Dairy cows	Growing steers	Mature steers	Dairy cows	Growing steers	Mature steers
Reticulo-rumen	6.03 $\times$ 10 ⁴	1.17 $\times$ 10 ⁵	2.04 $\times$ 10 ⁵	5.82 $\times$ 10 ⁸	4.54 $\times$ 10 ⁸	1.29 $\times$ 10 ⁹
Omasum	8.91 $\times$ 10 ⁴	1.82 $\times$ 10 ⁵	1.20 $\times$ 10 ⁵	4.13 $\times$ 10 ⁷	5.99 $\times$ 10 ⁷	1.26 $\times$ 10 ⁸
Abomasum	2.69 $\times$ 10 ³	5.75 $\times$ 10 ³	3.02 $\times$ 10 ⁴	7.80 $\times$ 10 ⁵	8.74 $\times$ 10 ⁵	7.61 $\times$ 10 ⁶
Small intestine	8.31 $\times$ 10 ²	2.18 $\times$ 10 ²	1.29 $\times$ 10 ⁴	8.95 $\times$ 10 ⁵	9.66 $\times$ 10 ⁴	6.45 $\times$ 10 ⁶
Caecum	7.08 $\times$ 10 ³	2.88 $\times$ 10 ⁴	1.41 $\times$ 10 ⁴	1.90 $\times$ 10 ⁶	4.87 $\times$ 10 ⁶	2.52 $\times$ 10 ⁶
Large intestine	3.31 $\times$ 10 ³	4.90 $\times$ 10 ⁴	4.27 $\times$ 10 ⁴	9.60 $\times$ 10 ⁵	7.40 $\times$ 10 ⁶	1.18 $\times$ 10 ⁷
Faeces	6.03 $\times$ 10 ³	2.10 $\times$ 10 ⁴	9.77 $\times$ 10 ⁴	NA	NA	NA

out of three growing steers, digesta from pre-gastric, gastric and post-gastric organs, and faeces all contained fungal populations. The distribution of anaerobic fungi throughout the digestive tract was similar for each group of animals. When expressed relative to digesta or faecal DM, the largest populations of anaerobic fungi were found associated with digesta contents from the rumen and omasum. These ranged from 6.03×10^4 t.f.u. (g DM)⁻¹ in the rumen digesta of dairy cows to 2.04×10^5 t.f.u. (g DM)⁻¹ in the rumen digesta of mature steers (Table 2). Within each group of animals, fungal populations associated with digesta from the gastric and post-gastric organs, and from faeces were at least an order of magnitude lower than those associated with digesta from the rumen and omasum. In three situations,

i.e. (a) digesta from the small intestines of dairy cows, (b) digesta from the abomasum of growing steers, and (c) digesta from the small intestines of growing steers, fungal t.f.u. (g DM)⁻¹ were up to three orders of magnitude lower than those detected in digesta from the comparative pre-gastric organs (Table 2).

When expressed as t.f.u. per organ, fungal populations were highest in the rumen and omasum, accounting for approximately 89% and 9% respectively of the anaerobic fungi in the entire digestive tract (Table 2). Fungal populations declined considerably (by up to 1000-fold) in the abomasum and small intestines and then remained unaltered or increased slightly in the caecum and large intestines (Table 2). Expressed as a percentage of the total, the fungal populations in the

Table 3. Fungal t.f.u. per g dry matter (DM) and the percentage survival of t.f.u. of the original fresh sample for dried digesta and faeces from growing steers

Each value represents the mean of three observations. The MPN enumerations used 10-fold dilutions and three replicates per dilution. The animals were 8-month-old steers with a mean live weight of 244 kg and were fed an *ad libitum* silage diet with fishmeal at 10% of the dry matter of the daily silage intake. Digesta and faeces were dried in air for 7 d at ambient room temperature in aluminium dishes. The 95% confidence limits represent the statistically possible lower and upper t.f.u. counts with a 95% degree of confidence.

Organ	Fungal t.f.u. (g DM) ⁻¹	95% Confidence limits		Percentage of original sample
		Lower	Upper	
Reticulo-rumen	4.57	1.62	1.02×10^1	0.004
Omasum	3.98×10^3	8.91×10^2	1.45×10^4	2.2
Abomasum	5.89×10^2	1.26×10^2	2.00×10^3	10.2
Small intestine	0.00	0.00	3.02×10^1	0.0
Caecum	2.19×10^3	4.78×10^2	8.91×10^3	7.7
Large intestine	3.31×10^3	1.12×10^3	1.74×10^4	6.8
Faeces	5.13×10^3	1.12×10^3	1.74×10^4	24.5

hindgut (caecum and large intestines) ranged from 0.5%, in dairy cows, to 1.0%, in mature steers, to 2.3%, in growing steers, of the population in the entire digestive tract. By summation of fungal t.f.u. in each organ, the mean population of anaerobic fungi in the entire digestive tract of the mature steers was calculated as 1.44×10^9 t.f.u.; populations in growing steers and lactating dairy cows were slightly lower at 5.27×10^8 and 6.28×10^8 t.f.u. per digestive tract, respectively.

Fungal populations in dried digesta and faeces after 7 d storage in air

When samples of digesta and faeces from growing steers were enumerated 7 d after the onset of drying and storage, all except digesta from the small intestines and two of the three replicate samples from the rumen were found to contain populations of anaerobic fungi. Fungal populations in dried digesta and faeces, expressed as t.f.u. (g DM)⁻¹ and as a percentage of those present in the original fresh samples, are shown in Table 3. Upon drying and storage, fungal populations in rumen digesta declined by 5 orders of magnitude [from 1.17×10^5 to 4.57 t.f.u. (g DM)⁻¹], representing a mean survival of 0.004% of the total population originally present (Table 3). Thus, if all of the digesta from the rumen had been dried, of the 4.54×10^8 t.f.u. initially present, only 1.82×10^4 would have survived the drying process. Fungal populations in omasal digesta declined by approximately 50-fold, representing a survival ability of 2.2% of the initial population (Table 3). Thus, the fungal population in the omasum was about 550 times more able to survive the drying process than that present in the rumen. Samples of digesta from the abomasum, hindgut

organs and from faeces, after drying, had values ranging from 6.8% to 24.5% of the original populations (Table 3). Thus, the fungi in digesta from these organs and the fungi in faeces were approximately 2000 to 6000 times more able to survive desiccation and storage than those present in rumen digesta, fungi in faeces having the greatest ability to survive.

Fungal populations in dried digesta and faeces after prolonged storage in air

Samples of digesta and faeces from mature steers were enumerated for anaerobic fungi, both when they were fresh and at various times after drying and storage. All fresh samples contained fungal populations (Table 2, Fig. 1). In all cases when survival occurred, the ultimate size of the surviving population was small in comparison to that present in the fresh sample. However, as in the previous experiment, the proportion of the surviving population increased in digesta from organs remote from the reticulo-rumen and in faeces.

Fungal t.f.u. in digesta from the small intestines, rumen and abomasum declined rapidly after drying such that viable populations were detectable only within the 1st, 7th and 22nd d of the experiment, respectively (Fig. 1). Omasal digesta retained a fungal population of approximately 1% of that present in the original fresh sample, but viable fungi were not detected after 57 d storage. Anaerobic fungi in digesta from post-gastric organs and faeces were more tolerant to desiccation and storage than those from the pre-gastric and gastric organs. Thus, the fungi present in caecal digesta were able to survive for up to 109 d, whereas those present in digesta from the large intestines and faeces maintained

Fig. 1. Fungal populations in fresh and dried digesta and faeces from mature steers, dried in air and stored for up to 252 d. Each value represents the mean of three observations. The MPN enumerations used 10-fold dilutions and 3 replicates per dilution. The 20-month-old steers had a mean live weight of 497 kg and were fed an *ad libitum* silage diet. Digesta and faeces were dried and stored in air for periods up to 252 d at ambient room temperature in aluminium dishes. (a) Reticulo-rumen; (b) omasum; (c) abomasum; (d) caecum; (e) large intestine; (f) faeces. Data for the small intestine are not presented as these samples did not have a viable anaerobic fungal population upon drying. Error bars represent the upper and lower 95% confidence limits of the t.f.u. enumerations.

detectable populations for 252 d of storage, when the experiment was terminated (Fig. 1).

Discussion

It is now accepted that anaerobic fungi have an important role in ruminal cellulolysis (Theodorou *et al.*, 1989, 1992). However, other than the information presented in this paper, and by Grenet *et al.* (1989), Milne *et al.* (1989), Davies *et al.* (1990) and Theodorou *et al.* (1990), little is known about fungal populations inhabiting parts of the digestive tract other than the rumen. This study has shown, for the first time, that anaerobic fungi are present throughout the entire digestive tract and provides the first enumeration of anaerobic fungi in these organs. The MPN procedure requires the enumeration tubes to be frequently observed using the light microscope (Theodorou *et al.*, 1990). From these observations the morphological characteristics of numerous fungal isolates, from both fresh and dried samples of digesta and faeces, were determined. Observations were limited to a cursory examination of zoospores and thalli and no attempt was made to segregate different isolates into genera and species. Nonetheless, the examination was sufficiently thorough to enable us to conclude that all samples contained representatives of the monocentric genera, *Neocallimastix*, *Piromyces* and *Caecomyces*. Thallus development was endogenous and the tapering rhizoids of *Neocallimastix* and *Piromyces* spp. were extensively

branched, whilst those of the *Caecomyces* were more limited, taking the form of bulbous spherical bodies or holdfasts. Zoospores were uninucleate, spherical, oval or waisted in appearance, with one (*Piromyces*, *Caecomyces*) or numerous (*Neocallimastix*) flagella. Anaerobic fungi belonging to the polycentric genera (e.g. *Orpinomyces*) were not observed during the course of this study, although they have been isolated from dried ruminant faeces on a separate occasion. For the animals studied, approximately 89% of the fungal population inhabited the rumen, whereas about 9% occupied the omasum and the remaining 2% was distributed between the organs of the midgut and hindgut (Table 2). Thus, the total population of anaerobic fungi in the rumen was considerably larger than that in the omasum and the midgut and hindgut organs. When fungal populations were related to the amount of DM in the sample, t.f.u. values in digesta from the rumen and omasum were approximately equal (Table 2). Compared with the pre-gastric organs, however, there was a marked decrease in fungal populations in digesta from the abomasum and small intestine, and these lesser populations remained unaltered or increased slightly in the hindgut organs and in faeces (Table 2).

Fungi present in the omasum and lower gut are probably carried out of the rumen on particulate material; this view is also held by Grenet *et al.* (1989) to explain their observations of the presence of anaerobic fungi in the duodenum and caecum of dairy cows. The dramatic decline in fungal populations in the midgut of

Fig. 2. Diagrammatic representation of the complete life cycle of anaerobic fungi. Cycle A is the vegetative cycle purported to occur in the rumen. Cycle B is the survival cycle which occurs when conditions become unfavourable for vegetative growth.

all animals tested, and subsequent increase in populations in the hindgut of some animals can be explained by the assumptions that (a) many zoospores and immature thalli may be killed during gastric digestion in the abomasum and that (b) some fungal growth and liberation of zoospores continues in the hindgut in much the same way as it does in the rumen.

Of the total population of anaerobic fungi in the digestive tract of growing steers (5.27×10^8), only 0.43% (2.29×10^6) were able to withstand drying in air for 7 d. Of these surviving fungi, the majority (99.9%) were located in parts of the digestive tract other than the rumen. Moreover, a much greater percentage (24.5%) of the fungi present in faeces were able to withstand the drying process than those present in any organ of the digestive tract (Table 3). These observations were confirmed in a similar experiment, in which anaerobic fungi from the digesta and faeces of mature steers were enumerated in both fresh samples and samples at various times after drying and storage. In this case, in addition to increasing survival with distance down the tract fungal populations in digesta from the hindgut and in faeces were able to survive for considerably longer periods than those present in the foregut and midgut regions (Fig. 1). These results serve to illustrate that fungi in the lower tract and in faeces, although lower in numbers, were more able to survive desiccation and exposure to air than those present in the rumen.

To account for our findings, we have amended the generally accepted fungal life cycle by inclusion of an additional survival stage as shown in Fig. 2. Cycle A is the vegetative cycle purported to occur in the rumen. Cycle B is an alternative route which is activated when conditions for vegetative growth become less favourable. We speculate that the survival stage is a thick walled

zoosporangium which is formed in a proportion of the anaerobic fungi leaving the rumen. This structure can then germinate when conditions become favourable, either in the hindgut if substrates are available, or after defaecation and re-introduction into a new host animal. Resistant cysts are common amongst aerobic chytrids and have recently been reported in the genus *Neocallimastix* (Wubah *et al.*, 1991b). These workers identified thick-walled zoosporangia which contained melanin and increased DNA levels, but which failed to germinate in batch culture.

The authors would like to thank Cathy King-Spooner, Mandy Fyrth, Barbara Williams and Lola Isac for their technical assistance. We would also like to thank Malcolm Gibb and Wendy Ivings for the provision of entire digestive tract samples. We thank the Agriculture and Food Research Council and the Science and Engineering Research Council for supporting this work.

References

- BAUCHOP, T. (1979). Rumen anaerobic fungi of cattle and sheep. *Applied and Environmental Microbiology* **38**, 148–158.
- DAVIES, D., THEODOROU, M. K. & TRINCI, A. P. J. (1990). Anaerobic fungi in the digestive tract and faeces of growing steers: evidence for a third stage in their life cycle. *Fourth International Mycological Congress, Abstracts*, IIE-226/3, edited by A. Reisinger & A. Bresinsky. Regensburg, Germany: Botanical Institute, University of Regensburg.
- GRENET, E., FONTY, G., JAMOT, J. & BONNEMOY, F. (1989). Influence of diet and monensin on the development of anaerobic fungi in the rumen, duodenum, cecum and feces of cows. *Applied and Environmental Microbiology* **55**, 2360–2364.
- LOWE, S. E., THEODOROU, M. K. & TRINCI, A. P. J. (1987). Isolation of anaerobic fungi from saliva and faeces of sheep. *Journal of General Microbiology* **133**, 1829–1834.
- MILNE, A., THEODOROU, M. K., JORDAN, M. G. C., KING-SPOONER, C. & TRINCI, A. P. J. (1989). Survival of anaerobic fungi in feces, in saliva, and in pure culture. *Experimental Mycology* **13**, 27–37.
- ORPIN, C. G. (1975). Studies on the rumen flagellate *Neocallimastix frontalis*. *Journal of General Microbiology* **91**, 249–262.
- ORPIN, C. G. (1977). The rumen flagellate *Piromonas communis*: its life-history and invasion of plant material in the rumen. *Journal of General Microbiology* **99**, 107–117.
- ROSS, G. J. S. (1987). MLP; Maximum likelihood program. Harpenden: Rothamstead Experimental Station.
- THEODOROU, M. K., LONGLAND, A. C., DHANOA, M. S., LOWE, S. E. & TRINCI, A. P. J. (1989). Growth of *Neocallimastix* sp. strain R1 on Italian ryegrass hay: removal of neutral sugars from plant cell walls. *Applied and Environmental Microbiology* **55**, 1363–1367.
- THEODOROU, M. K., GILL, M., KING-SPOONER, C. & BEEVER, D. E. (1990). Enumeration of anaerobic chytridiomycetes as thallus forming units: a novel method for the quantification of fibrolytic fungal populations from the digestive tract ecosystem. *Applied and Environmental Microbiology* **56**, 1073–1078.
- THEODOROU, M. K., LOWE, S. E. & TRINCI, A. P. J. (1992). Anaerobic fungi and the rumen ecosystem. In *The Fungal Community: Its Organisation and Role in the Ecosystem*, 2nd edn, pp. 43–72. Edited by G. C. Carroll & D. T. Wicklow. New York: Marcel Dekker.
- WUBAH, D. A., FULLER, M. S. & AKIN, D. E. (1991a). Isolation of monocentric and polycentric fungi from the rumen and feces of cows in Georgia. *Canadian Journal of Botany* **69**, 1232–1236.
- WUBAH, D. A., FULLER, M. S. & AKIN, D. E. (1991b). Resistant body formation in *Neocallimastix* sp., an anaerobic fungus from the rumen of a cow. *Mycologia* **83**, 40–47.