SHORT COMMUNICATIONS

Aspergillus fumigatus and *Sporormia minima* Isolated from the Rumen of Sheep

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The toxic metabolites of the micro-fungi are associated with many problems in the food-producing industries. The role of isolates of *Aspergillus flavus* that produce aflatoxin in the toxicity of feed-stuffs derived from ground nuts is well known (Paget, 1954; Wogan, 1966) and there is some evidence that metabolites of *Pithomyces chartarum* are implicated in the aetiology of diseases of ruminants in Australia and New Zealand and possibly in South Africa and the southern United States (Dingley, Done, Taylor & Russell, 1962; Mortimer & Taylor, 1962). Bilai & Pidoplichko (1962) suggested that the fungus *Absidia ramosa*, isolated aerobically from the rumen contents of diseased animals was a factor in haematuria in cattle in the Ukraine. This, to our knowledge, is the first suggestion that the colonization of the rumen by microfungi has important implications in the growth and health of ruminants; although the presence of yeasts in the rumen has been known for some time (Clarke & diMenna, 1961). In northern Nova Scotia the growth rate of sheep in summer and autumn is extremely slow (Calder, Nicholson & Cunningham, 1962), and whilst investigating this problem from a microbiological point of view we have observed the presence of fungi among the rumen flora. Since this appears to be of biochemical, microbiological and agricultural interest we report our preliminary results here.

METHODS

Female Shropshire sheep, 10 months old, were maintained at the rate of 10 to the acre on good pasture. Before sampling, they were transferred into pens with concrete floors, where they were kept for 2 hr. After this time samples (30 ml.) of rumen fluid were aspirated by means of an oesophageal tube into sterile 50 ml. containers. The tube was retracted to flush the container with rumen gas. The containers were capped and transferred to the laboratory (2 min.). The filtered rumen fluid was diluted (1/10) with the diluent described by Kurihara, Eadie, Hobson & Mann (1968), omitting glucose, under strict anaerobic conditions and the diluted rumen fluid (1 ml.) transferred to rumen-fluid agar (prepared from freshly collected rumen liquor from donor animals grazing the same pasture; Kurihara *et al.* 1968) at 50°, 5 mm. thick in Petri dishes. The cultures were exposed to air while the agar set (< 10 min.), then

they were placed in large desiccators which were evacuated and then filled with oxygen-free carbon dioxide and incubated at 39°.

Samples (0.1 ml.) from the diluted rumen fluid were also used to inoculate 100 ml. medium containing dehydrated malt extract (Difco, 2 g.), agar (2 g.) and rose bengal (3 mg.). These cultures were incubated aerobically at 25°. The malt medium inoculated with the diluted rumen fluid (0.2 ml.) was also incubated aerobically at 39°.

RESULTS

While counting viable organisms in a culture of a rumen fluid sample from sheep No. 33 on the medium described by Kurihara *et al.* (1968), our colleague Miss D. E. Hannah noticed a fungal colony at a dilution of 10⁷. A number of subcultures were attempted from this colony on both 2 % (w/v) malt agar and on rumen-fluid agar, at 25° and at 39°, aerobically and anaerobically. In only one instance was subculture successful and this was on rumen-fluid agar incubated anaerobically at 39°. The growth of the fungus was mycelial.

Table 1. *Fungal colonies isolated on 30 October 1968 from the rumen contents of sheep*

Sheep no.	No. of colonies/ml. rumen fluid			Identified organisms growing anaerobically
	Isolated aerobically* (25°)	Isolated aerobically* (39°)	Isolated anaerobically† (39°)	
5	1400	110	4	<i>Sporormia minima</i>
6	4200	390	21	<i>S. minima</i>
22	3600	290	30	<i>S. minima</i>
33	8300	500	57	<i>S. minima</i> and <i>Aspergillus fumigatus</i>
42	2400	340	2	<i>A. fumigatus</i>
45	5100	460	60	Not identified
48	2100	310	18	<i>A. fumigatus</i> and <i>S. minima</i>
54	1400	280	9	Not identified

The temperatures given are those at which the organisms were cultured.

* On malt agar medium.

† On rumen-fluid agar medium

During the following 6 weeks microscopic examination of samples of rumen fluid from sheep grazing the same areas revealed fungal spores and mycelial fragments. An attempt was made to isolate these fungi and to obtain approximate values for the numbers of viable fungi present. We were unable to do this under strict anaerobic conditions because of the rapid growth of bacteria, but the exposure to air during preparation of the cultures was sufficient to reduce bacterial growth and permit growth of fungi. The organisms were therefore cultivated aerobically at 25° and 39° and, after exposure to air, anaerobically at 39°. The results obtained on rumen fluid samples from eight sheep are given in Table 1. For the aerobic cultures ten samples of each rumen fluid were incubated at 25° and a similar number at 39°. The figures given in the Table are summations of all the colonies counted (divided by 2 in the case of those incubated at 39°). In the case of the anaerobic cultures only four plates

were inoculated with each sample, thus these results merely indicate that fungi which grew anaerobically at 39° could be isolated from each animal. During the following winter the same animals were maintained on chopped hay and oats, and 5 months after the above results were obtained we were unable to demonstrate the presence of fungi having the ability to grow anaerobically at 39°.

The colonies that grew anaerobically at 39° could not be identified, as only vegetative growth was observed. When they were transferred to malt agar, in air at 25°, growth occurred in all cases and *Aspergillus fumigatus* Fres. was identified. *Sporormia minima* Auersw. was identified by further subcultivation on Leonian's (1925) medium enriched with 0.3 % (w/v) yeast extract. Several species remain to be identified. Hence the fungi isolated anaerobically were all facultative anaerobes and differed from the organism originally observed.

DISCUSSION

Aerobic fungi might be expected in the rumen because of their ubiquitous presence on animal feed-stuffs and the ability of some of these organisms to grow anaerobically is perhaps not surprising. However this does not appear to have been reported previously and we have been unable to isolate fungi capable of anaerobic growth from sheep fed indoors on hay and oats. Some of our anaerobic isolates await identification, hence comparison between populations of fungi obtained, for example, from spore trap samples of grazed herbage and those in the rumen is difficult. The results must therefore be regarded as preliminary and further work is in progress.

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