Rumen microbial ecosystem

D. N. Kamra

Microbiology Section, Centre of Advanced Studies in Animal Nutrition, Indian Veterinary Research Institute, Izatnagar 243 122, India

The inhabitants of the rumen microbial eco-system, a complex consortium of different microbial groups living in symbiotic relationship with the host, act synergistically for the bioconversion of lignocellulosic feeds into volatile fatty acids which serve as a source of energy for the animals. The constraints, imposed by the host and the feed consumed by the animal, under which these microbes have to function, have been discussed. The eco-system is specialized and buffered in a narrow range of pH, which helps the animal to maintain a very well stabilized eco-system which is not disturbed by the incoming microbial contaminants into the fermentation sac (rumen) through feed and water intake. The microbial ecosystem is well studied for the rumen of domesticated animals like cattle, sheep and goat, but it is poorly studied in buffalo and wild ruminants. The necessity to use molecular biology techniques for identification and characterization of rumen microbes has been emphasized in this review.

IN tropical countries of the world, the ruminants are fed on lignocellulosic agricultural by-products like cereal straws, stovers, sugarcane bagasse, tree foliages and cakes of oil seeds like groundnut, cotton, mohua, neem and mustard. The efficiency of ruminants to utilize such a wide variety of feeds is due to highly diversified rumen microbial eco-system consisting of bacteria (10^{10}–10^{11} cells/ml, representing more than 50 genera), ciliate protozoa (10^4–10^6/ml, from 25 genera), anaerobic fungi (10^3–10^5 zoospores/ml, representing five genera) and bacteriophages (10^8–10^9/ml)[1]. These numbers might even be larger as majority of them are non-culturable. The synergism and antagonism among the different groups of microbes and even among different genera of the same group is so diverse and complicated that it is difficult to quantify the role played by any particular group of microbes present in the rumen. The net result of these reactions going on in the rumen is responsible for the bioconversion of feed into such form that is utilizable by the animal as a source of energy (short chain volatile fatty acids). These microbes survive in the rumen under different constraints which may be either natural or feed associated as some of the feeds contain a significant amount of anti-nutritional factors, which sometimes limit the growth of some of these natural microbial inhabitants.

Constraints in the rumen

Natural environmental constraints

The microbial eco-system of the rumen is stable and at the same time dynamic. The eco-system is stable as it is well established and has been performing the function of bioconversion of feed into volatile fatty acids. In a healthy ruminant the contamination of the eco-system does not occur in spite of the fact that millions of microbes invade the rumen everyday through feed, drinking water and air. The eco-system is dynamic as the microbial population changes considerably on change of diet so as to adapt it to the new feed ingredients. This happens because the rumen microbes are adapted to survive in a set of constraints prevalent in the rumen and any contaminant which cannot survive these constraints is eliminated. The major environmental constraints are anaerobiosis, high buffering capacity and osmotic pressure and saprophytic competition among the microbes for their survival.

The anaerobiosis inside the rumen is one of the major constraints in the rumen eco-system, which helps in conserving the energy ultimately to be used by the host animal. The amount of energy released by electron transfer reaction depends upon the terminal electron acceptor. In aerobic microbes, the terminal electron acceptor is oxygen and the release of energy in the form of ATP is much higher compared to energy released when the terminal electron acceptor is an organic compound as in the case of anaerobic bacteria[2]. The anaerobic conditions in the rumen are maintained by gases generated during fermentation, e.g. carbon dioxide, methane and traces of hydrogen. Some of the oxygen entrapped in the feed consumed by the animal is utilized by the facultative anaerobes present in the rumen and thus a perfect anaerobic condition is generated and maintained. Therefore, only the microbes which are able to tolerate such a low redox potential (–350 mV) are able to survive in the rumen and the rest are eliminated from the system. High buffering capacity and osmotic pressure too limit the growth of invading microbes. Some of the rumen microbes produce anti-microbial compounds which limit the growth of other microbes present in the eco-system[3,4].

Feed-associated constraints

The livestock are fed mainly on agro-industrial by-products, which contain a lot of anti-nutritional factors which may

e-mail: dnkamra@rediffmail.com

act as inhibitors for some of the rumen microbes. The anti-nutritional compounds like tannins, lignins, saponins, mimosine, etc. are synthesized in the plants to protect them against the invading microbes. Therefore these compounds have anti-microbial activity. Therefore when consumed by the animals, these compounds limit the growth of different types of microbes (useful and undesirable) in the rumen.

Tannins. Tannins are phenolic compounds with molecular weight ranging from 500 to 3000 Da, which are water soluble, able to precipitate proteins, alkaloids and gelatin. Tannins are usually secondary metabolites of plants which are not involved in primary metabolic pathways of plants. Based on the differences in their chemical structure and relative ratios of different phenolic monomers, tannins are classified into two groups, e.g. hydrolysable and condensed tannins. The hydrolysable tannins are mostly the esters of gallic acid (gallotannins) or ellagic acid (ellagitannins) with a sugar core (usually glucose), which are readily hydrolysed by acids or enzymes into monomeric products, on the other hand the condensed tannins are polymeric proanthocyanidins.

Tannins are most effective against the fibre-degrading bacteria. McSweeney *et al.*[5] observed that in animals fed tannin rich *Calliandra calothyrsus*, the population of *Ruminococcus* spp. and *Fibrobacter* spp. was reduced considerably, but fungi, protozoa and proteolytic bacteria were less affected by this diet. Sotohy *et al.*[6] reported that the number of total bacteria in the rumen of goats decreased significantly when the animals were fed tannin-rich plant (*Acacia nilotica*) and the decrease in the numbers was directly proportional to the level of this feed in the diet. Condensed tannins from the leaves of sainfoin (*Onobrychis viciifolia*) inhibited growth and protease activity in *Butyrivibrio fibrisolvens* A38 and *Streptococcus bovis* 45S1, the growth of *Prevotella ruminicola* B14 and *Ruminobacter amylophilus* WP225 was not much affected[7].

It was perhaps for the first time that Brooker *et al.*[8] isolated a Gram-positive facultative anaerobe which could grow in a medium containing 2.5% tannic acid or condensed tannins from the rumen liquor of feral goats browsing on *Acacia* spp leaves. The bacterium was named as *Streptococcus caprinus*. It was not a major inhabitant of the rumen and was present at a population density of 2×10^6 per ml of rumen fluid.

Odenyo and Osuji[9] isolated three strains of tannin-tolerant bacteria from sheep, goat and an antilope and observed that the isolates could tolerate tannins up to 8 g/litre in the medium. Their growth increased when soluble carbohydrates are included in the growth medium. Nelson *et al.*[10] isolated six strains from the rumen contents of Sardinian sheep (*Ovis aries*), Honduran and Colombian goats (*Capra hircus*), white tailed deer (*Odocoileus virginianus*) and Rocky Mountain elk (*Cervus elaphus nelsoni*).

Four of the isolates belonged to the genus *Streptococcus*, most closely related to *S. bovis* and *S. gallolyticus*.

The ruminants which continuously feed upon diets rich in tannins usually develop a microflora which is tolerant to high tannins. The feral goats and camel fed on *Acacia* and *Calliandra calothyrsus*, which contain high level of tannins, are capable of tolerating tannins in diet due to the presence of high numbers of tannin-resistant bacteria like *Streptococcus caprinus* and *Selenomonas ruminantium* K2 (ref. 11). The other isolated bacteria belong to the genera *Butyrivibrio* sp. and *Lactobacillus* sp. As confirmed by 16S rDNA restriction fragment length polymorphism (RFLP) analysis, *S. caprinus* and *S. gallolyticus* have been shown to be synonyms based on their 16S rRNA sequence similarity (98.3%) and DNA–DNA homology (>70%) and this bacterium has been preferred to be named as *Streptococcus gallolyticus*[12].

Feral goats fed mulga (*Acacia aneura*) were more resistant to tannins than sheep fed grass, but the capability to digest or tolerate tannins in diet increased when rumen flora was trans-inoculated into the rumen of sheep, indicating that goats contain microbial populations which could degrade tannins efficiently[13]. In another study in Indonesia, Wiryawan *et al.*[14] isolated five types of tannin-degrading bacteria, which could tolerate 3% tannic acid or 1% condensed tannins in their growth medium and reduce the tannin content by 52% in 72 h of growth. Inoculation of these bacteria at the rate of 3×10^{11} cfu into the rumen of unadapted goats could help in improving feed digestibility and live weight gain in the animals.

As discussed above tannins do have harmful effect on rumen microbes, but on prolonged feeding some of the tannin-degrading bacteria proliferate and increase in numbers and make the animals more tolerant to higher levels of tannins in their diet.

Saponins. Saponins are steroidal sapogenin covalently linked to oligosaccharide moiety. The saponins cause haemolysis of red blood cells perhaps by increasing the permeability of the plasma membrane and inhibit smooth muscle activity[15]. The saponins of lucerne origin had a detrimental effect on rumen fermentation and caused a reduction in total volatile fatty acid production and acetate to propionate ratio from 1.93 to 1.37 in the presence of 1% saponin in the medium[16]. The rumen microbes are able to deglycosylate the saponins to release the steroid moiety, which affects rumen fermentation. *Yucca schidigera* extract has been found to alter rumen fermentation favourably, increase animal growth and milk production[17,18]. The *yucca* extract is also inhibitory for some of the rumen ciliate protozoa and bacteria[19]. Wang *et al.*[20] also confirmed the anti-protozoal activity of *yucca* exract in RUSITEC experiments and reported an increase in the protease activity of the rumen microbes. Thalib *et al.*[21] studied the effect of saponins of *Sapindus rarak* fruit on rumen microbes of sheep and reported that the methanol extract of

seeds caused a 57% reduction in the number of protozoa and 69% increase in bacterial population which resulted in improved feed conversion efficiency and better gain in body weight of the animals.

Mimosine. A tropical browse leguminous plant, *Leucaena leucocephala* contains a toxic compound, mimosine, which restricts its nutritional use in domestic ruminants and monogastric animals. Some of the rumen microbes are able to degrade mimosine and its pyridine diol derivatives, i.e. 3,4-dihydroxypyridine and 2,3-dihydroxypyridine (3,4-DHP and 2,3-DHP). 3,4-DHP is a potent goitrogen of the thiouracil type, which is excreted in the urine. The Hawaiin goats fed on *Leucaena* fodder were found to contain a microflora which was able to degrade 3,4-DHP. The trans-inoculation of mixed inoculum from these goats to Australian ruminants sensitive to mimosine toxicity resulted in the transfer of the capability of degrading DHP completely and efficiently[22]. Allison *et al.*[23] isolated a bacterium capable of degrading DHP from the rumen of goats resistant to mimosine toxicity and named it as *Synergistes jonesii*. Four strains of *S. jonesii* have been isolated and all of them are Gram-negative, rod-shaped and obligate anaerobic. The inoculation of this culture in the rumen of mimosine intolerant animals extends protection against mimosine[24], indicating that some of the microbes are able to fill a specific niche in the rumen depending upon the availability of a specific component for its survival in the rumen. The bacterium isolated is an obligate anaerobe, Gram-negative, non-motile oval rod and non-spore forming. It can ferment 2,3- and 3,4-DHP, arginine and histidine, but carbohydrates are not fermented. An oligonucleotide probe which targets specific region of 16S rRNA, has been developed. This will help in studying the population of this microbe, its establishment in the new environment when inoculated and its colonization[25].

Nitrate–nitrite. Certain crops like oat, wheat, barley, etc. accumulate nitrates up to the concentration which may be fatal to the animal. The nitrate entering the rumen gets metabolized into nitrite which is absorbed into the blood rapidly and oxidizes oxyhaemoglobin to methemoglobin, thus reducing the oxygen-carrying capacity of red blood cells. Though some nitrite is metabolized further into ammonia, depending upon its level toxicity may occur. The rate of metabolism of nitrate and nitrite depends on a number of factors like particular microbial population, enzyme activity (nitrate reductase), pH, H donor, etc. Takahashi *et al.*[26] reported that the rate of nitrate and nitrite reduction was faster when the animals were fed on the diet rich in readily available carbohydrates, because the anaerobic microbes use fermentation products of carbohydrates like formate, pyruvate, lactate, glycine, etc. as a substrate for the reduction reaction. Five groups of bacteria have been isolated from a sheep adapted to nitrate[27]. Two groups belonged to selenomonads, third group could not be classi-

fied, fourth group identified as *Anaerovibrio* spp and the fifth was lactate-fermenting short rods.

Microbial diversity of rumen

Bacteria

Ruminants in India are fed mainly on lignocellulosic agricultural by-products which are rich in cellulose, hemicellulose, lignin, starch, protein and a very small quantity of oils. The rumen harbours various types of bacteria which are active in degradation of these components of the feed (Table 1). The interaction among themselves and with other microbial groups in the rumen are also responsible for synergistic effect on the production of volatile fatty acids and microbial proteins in the rumen.

Some of the common features of bacteria found in the rumen of animals fed on high roughage diet are as follows:

- Majority of the bacteria are Gram-negative. The number of Gram-positive bacteria tends to increase on increasing high energy diets in the ration.
- Most of the bacteria are obligate anaerobes. Some of them are so sensitive to oxygen that these are killed on exposure to oxygen. A few rumen bacteria require a very low redox potential (indicating a high degree of anaerobiosis) and grow at a redox potential lower than –350 mV.
- The optimum pH for the growth of rumen bacteria lies between 6.0 and 6.9.
- The optimum temperature is 39°C.
- The bacteria can tolerate a considerably higher level of organic acids without affecting adversely their metabolism.

The bacteria isolated from the rumen of Indian cattle and buffalo were represented by Ruminococci to the extent of 50–94% on feeding of 15% sugarcane molasses and 2–3% urea to the animals[28]. Some of the cellulose-degrading bacteria isolated from the rumen of buffalo include *Fibrobacter succinogenes, Ruminococcus albus* and *R. flavefaciens* as reviewed by Jalaludin *et al.*[29].

Berseem supported maximum number of cellulose-degrading bacteria and were represented by *Ruminococcus albus* and *R. flavefaciens* (59.8%). *Bacteroides succinogenes* (*Fibrobacter succinogenes*) (19.2%), *Butyrivibrio fibrisolvens* (11.1%), *Clostridium lochheadii* (3.8%) and *C. longisporum* (1.3%)[30]. The cellulose-degrading bacteria get stimulated (57.4% higher numbers than that in control) when buffaloes are fed *Saccharomyces cerevisiae* in their diets[31] which might be due to release of some un-identified micro nutrients by the yeast which are essentially required by the cellulose-degrading bacteria. Recently Sahu *et al.*[32] isolated a cellulose degrading bacteria from the faeces of chinkara (*Gazella gazella*) and identified it as *Ruminococcus flavefaciens.*

Table 1. Bacterial diversity of the rumen microbial ecosystem of domestic and wild animals

Substrate	Bacteria	Ref.
Bacteria active in carbohydrate utilization		
Cellulose	*Fibrobacter succinogenes*	100–101
	(*Bacteroides succinogenes*)	
	Ruminococcus flavefaciens	41, 102–104
	Ruminococcus albus	
	Clostridium cellobioparum	105
	Clostridium longisporum	106
	Clostridium lochheadii	
	Eubacterium cellulosolvens	107, 108
	(*Cillobacterium cellulosolvens*)	
Hemicellulose	*Butyrivibrio fibrisolvens*	109, 110
	Prevotella ruminicola	111
	(*Bacteroides ruminicola*)	
	Eubacterium xylanophilum,	112
	E. uniformis	
Starch	*Streptococcus bovis*	113
	Ruminobacter amylophilus	114, 115
	(*Bacteroides amylophilu*)	
	Prevetella ruminicola	111
	(*Bacteroides ruminicola*)	
Sugars/dextrins	*Succinivibrio dextrinosolvens*	110
	Succinivibrio amylolytica	107
	Selenomonas ruminantium	116
	Lactobacillus acidophilus, L. casei,	37, 117
	L. fermentum, L. plantarum, L. brevis,	
	L. helveticus	
	Bifidobacterium globosum, B. longum,	118
	B. thermophilum	
	B. ruminale	119
	B. ruminantium	120
Pectin	*Treponema saccharophilum*	121
	Lachnospira multiparus	110, 122
Bacteria active in nitrogen utilization		
Protein degraders	*Prevotella ruminicola*	
	Ruminobacter amylophilus	
	Clostridium bifermentans	123
Urea hydrolysers	*Megasphaera elsdenii*	124, 125
Other bacteria		
Acid utilizers	*Megasphaera elsdenii*	126, 127
	(*Peptostreptococcus elsdenii*)	
	Wollinella succinogenes	128
	(*Vibrio succinogenes*)	
	Veillonella gazogenes	129
	(*Veillonella alcalescens,*	
	Micrococcus lactolytica)	
	Oxalobacter formigenes	130
	Desulphovibrio desulphuricans	131, 132
	Desulphatomaculum ruminis	133
	Succiniclasticum ruminis	134
Lipolytic bacteria	*Anaerovibrio lipolytica*	135
Acetogenic bacteria	*Eubacterium limosum*	136
	Acetitomaculum ruminis	137
Tannin degraders	*Streptococcus caprinus*	8
	Eubacterium oxidoreducens	138
Mimosine degraders	*Synergistes jonesii*	23
Methanogenic archea	*Methanobrevibacter ruminantium*	139, 140
	Methanobacterium formicicum	
	Methanosarcina barkeri	141, 142
	Methanomicrobium mobile	143
Mycoplasma	*Anaeroplasma bactoclasticum*	144
	Anaeroplasma abactoclasticum	145

Archeaea

Seven different species representing five genera of methanogens have been reported from the rumen of different animals, i.e. *Methanobacterium formicicum, Methanobacterium bryanti, Methanobrevibacter ruminantium, Methanobrevibacter smithii, Methanomicrobium mobile, Methanosarcina barkeri* and *Methanoculleus olentangyi*[33,34]. Methanogens are present in the rumen in large numbers varying from 10^7 to 10^9 cells/ml of rumen liquor depending upon the type of diet given to the animals, especially the fibre content in the ration. The methanogens play a vital role in the rumen of scavenging molecular hydrogen generated during rumen fermentation, thereby making rumen fermentation a continuous process, but this leads to a significant loss of gross energy consumed by the animals.

There is a close association between entodiniomorphid protozoa and some of the bacteria, which attach to the pellicle of protozoa. Eleven species of entodiniomorphid protozoa have been found to have adhered methanogens like *Entodinium longinucleatum, Eudiplodinium maggii, Entodinium bursa* and *Eremoplastron bovis*. The methanogens attach themselves with the ciliate protozoa to get a constant supply of hydrogen. On pumping hydrogen in the rumen, the methanogens get detached from the protozoa[35].

Protozoa

The rumen protozoa were detected in domestic animals as early as nineteenth century by Gruby and Delafond[36]. Not much work was done for several decades after their first report in the rumen. It was only after 1920 that the researchers paid any significant attention towards the identification, morphology and biochemical functions of protozoa in the rumen. At present a lot of information has been generated and compiled in the form of research papers, reviews, bulletins, books, symposium proceedings, etc.[1,37–40].

The ciliate protozoa of the rumen have been classified into two groups depending upon their morphological characteristics, i.e. holotrich and entodiniomorphid protozoa[37]. Alternatively these can also be classified as soluble sugar utilizers, starch degraders and lignocellulose hydrolysers. The holotrich protozoa are represented by 15 different genera in the rumen of different animals. Among these genera, *Isotricha, Dasytricha, Buetschlia* and *Charonina* are some which are widely distributed in the rumen of domestic and wild ruminants and hind gut fermentors[41,42] (Table 2). Some of the non-ruminant pre-gastric fermentors like camel and hippopotamus also have a large number of holotrich protozoa[43,44].

The enzymatic profile of holotrich protozoa indicates that they have amylase, invertase, pectin esterase and polygalacturonase in sufficiently large quantities for using starch, pectin and soluble sugars as energy source[45–48]. The en-

Table 2. Protozoal diversity of the rumen microbial ecosystem of domestic and wild animals

Protozoa	Host	Ref.
Holotrich protozoa		
Isotricha prostoma	Sheep	146
	Zebu cattle	147
	Blackbuck	148
	Water buffalo	54
	Red deer	149
	Goat	150
I. intestinalis	Cattle	151
	Bison	146
	Mouse deer	147
	Red deer	149
Dasytricha ruminantium	Sheep	152
	Blackbuck	148
	Water buffalo	54
Oligoisotricha bubali	Water buffalo	59
	Cattle	61
Entodiniomorphid protozoa		
Entodinium bovis	Yugoslavian cattle	153
	Zebu cattle and water buffalo	154
E. bubalum	Water buffalo	59
E. bursa	Cattle sheep and goat	92
E. caudatum	Cattle sheep and goat	155
E. chatterjeei	Indian goat	150
	Water buffalo	154
E. longinucleatum	Cattle, water buffalo	154
Diplodinium dendatum	Widely distributed	151
D. indicum	Indian cattle	156
Eremoplastron asiaticus	Indian cattle	156
E. bubalus	Brazillian cattle and water buffalo	54
Eudiplodinium maggii	Widely distributed	157
Ostracodinium trivesiculatum	Cattle and water buffalo	54
Polyplastron multivesiculatum	Cattle, sheep, goat, blackbuck	148, 158–160
Metadinium medium	Cattle	161
	Water buffalo	54
	African reedbuck	162
Epidinium caudatum	Cattle, sheep, goat	161
Ophryoscolex caudatus	Widely distributed	150, 158, 161
Caloscolex camelicus	Dromedary camel	163

zymes responsible for cellulose and hemicellulose degradation have also been reported in the holotrich protozoa but the levels are very low compared to those present in the entodiniomorphid protozoa[49].

The microbiology of rumen of cattle and sheep has been extensively studied and reviewed by many workers from time to time[37,50], but studies on rumen microbes of buffalo are limited[29,51,52] and the experiments on relative microbiology of cattle and buffalo on similar diets and same environmental conditions are rare. The total number of ciliate protozoa is lower in buffalo than that in cattle, but the ciliate protozoa represented in both the species of animals included *Entodinium, Diplodinium, Eremoplastron, Eudiplodinium, Elytroplastron, Metadinium, Ostracodinium, Epidinium, Dasytricha* and *Isotricha*[53].

Dehority[54] studied the ciliate protozoa in the rumen of Brazilian water buffalo and found that 49 species of ciliate protozoa were present, out of which eight species were described for the first time. Four new species belonged to the genus *Diplodinium (Ostracodinium)*, i.e. *O. brazili, O. esalqum, O. nucleolobum* and *O. tiete*; three new species of *Entodinium*, i.e. *E. ciculum, E. spinonucleatum* and *E. triangulum* and one new species of *Diplodinium (Eudiplodinium) E. bubalus* have also been reported. In addition to the above, new species of *Entodinium, Diplodinium, Ostracodinium, Eudiplodinium, Elytroplastron, Epidinium, Isotricha, Dasytricha, Charonina* and *Buetschlia* have also been found in the rumen of buffalo which are commonly found in other domestic and wild ruminants. In another study 17 species of ciliate protozoa have been detected in Malaysian water buffalo and 15 in Kedah Kelantan cattle[55]. There was no difference in the total number of ciliates in two species of animals. *Metadinium ypsilon* and *Ostracodinium trivesculatum* have been found to be present in both the species, but other species reported in Malaysian ruminants are similar to those reported in other tropical and temperate ruminants[56].

Dumag[57] studied the ciliate protozoa in the rumen of Philippine carabaos (*Bubalus bubalis*) and found 59 different species of ciliates, out of which 21 belonged to *Entodinium*, ten to *Diplodinium*, eight to *Epidinium*, four to *Ostracodinium* and *Eremoplastron*, three to *Metadinium* and *Eodinium* and one each to *Enoploplastron, Diploplastron* and *Eudiplodinium*. Three species belonged to holotrich protozoa, e.g. *Isotricha intestinalis, I. prostoma* and *Dasytricha ruminantium*. Most of these species have also been reported in the rumen of cattle[37]. *Metadinium medium* has been reported to be present only in the rumen of buffalo[29]. Four new species of ciliate protozoa which have been reported to be present in the rumen of buffalo are *Entodinium ogimotoi, E. bubalum, E. fujitai* and *E. tsunodai*[58]. *Oligoisotricha bubali* has been considered to be restricted only in buffalo rumen[59,60], but it has also been detected in the rumen of cattle in the areas of Tennesse, USA. This protozoan has been observed in low numbers (<1% of total protozoa) to very high numbers (>70% of total protozoa) in the rumen of cattle fed different rations[61]. Corn silage-concentrate diet has been found to stimulate the growth of this protozoan in the rumen of cattle.

Eight genera of ciliate protozoa have been observed in the rumen of cattle and buffalo fed on wheat straw and concentrate mixture, e.g. *Isotricha, Dasytricha, Metadinium, Diplodinium, Eudiplodinium, Ophryoscolex, Entodinium,* and *Epidinium. Oscillospira guillermondii* was always found in the rumen of buffalo[62]. The protozoa production rate, estimated by use of ^{35}S as indicator, showed that it was lower in buffalo (25.4–27.5 mg/min) than that in the rumen of cattle calf (30.5 mg/min)[63]. There was no difference in the numbers of ciliate protozoa in the rumen liquor of cattle and buffalo[62,64].

Effect of defaunation on animal performance

- There is no pH stabilization in the rumen in the absence of ciliate protozoa and therefore, a low pH is always observed[65].
- There is an increase in the level of lactic acid and propionic acid in the rumen liquor[66,67].
- Ammonia nitrogen decreases significantly on defaunation[68].
- Methanogenesis is reduced considerably in the absence of ciliate protozoa[69,70].
- There is a significant increase in the numbers of bacteria and fungi in the rumen liquor when protozoa are eliminated, perhaps to take over the several general functions of protozoa in the rumen.
- There is an increased feed conversion efficiency on some diets, especially the high roughage diets[71,72]. This might be due to the absence of any specific function of ciliate protozoa on high roughage based diet.

Fungi

The flagellates were observed in the rumen as early as 1910, but were believed to be flagellate protozoa[73,74] and were placed in the genera *Callimastix, Sphaeromonas, Oikomonas*, etc. These flagellates were discovered as fungi for the first time in mid-seventies by Orpin[75], who identified it as *Neocallimastix frontalis*. The flagellate zoospore grew into a mycelium which in turn transformed into a reproductive stage of rhizoids bearing zoosporangium. This was confirmed to be a true fungus by the presence of chitin in its cell wall by Orpin[76]. The isolated organism was similar in life cycle and morphology to a chytridiomycete fungus, but this was a first report that it was a strict anaerobic fungus. Several strains of anaerobic fungi have been reported in the rumen of different ruminant species (Table 3).

These obligate anaerobic fungi, found in the rumen and other parts of the gastro-intestinal tract of herbivorous animals, have an active and positive role to play in fibre degradation as evidenced by the presence of different en-

Table 3. Fungal diversity of the rumen microbial ecosystem of domestic and wild animals

Fungus	Source of isolation	Ref.
Neocallimastix frontalis	Cow	76
N. patriciarum	Sheep	164
N. hurleyensis	Cattle	165
Sphaeromonas communis (*Caecomyces communis*)	Cattle	166, 167
Caecomyces equi	Horse	168
Orpinomyces bovis	Cattle	169
Anaeromyces mucronatus (*Ruminomyces mucronatus*)	Cattle	170
Ruminomyces elegans	Cattle	171
Piromyces communis, *Piromyces mae,* *Piromyces dumbonica*	Horse, elephant	172

zymes involved in fibre degradation[77,80]. The recent experiments conducted in the author's laboratory have shown that on removal of fungi from the rumen content, there is a significant reduction in *in vitro* gas production and degradation of fibrous feeds, indicating a positive role played by fungi in fibre degradation. The enzyme profile of various fungi studied indicates that a wide variety of enzymes required for lignocellulose degradation are excreted and it is confirmed by the scanning electron microscopic studies that these fungi prefer to get attached to the most lignified tissues of plant feed material[81]. The fibre-based diets stimulate the fungal growth in the rumen of buffalo[82] in comparison to diets rich in easily fermentable carbohydrates. Pelletted diets usually have a shorter transit time through the gastro-intestinal tract, therefore do not support the growth of anaerobic fungi in the rumen. Large quantities of soluble sugars inhibit the germination of zoospores on plant tissues[83]. This might be due to lowering of pH of the rumen liquor in the presence of high sugar concentration, which inhibits the production of zoospores in the rumen[84]. Because of the presence of different enzymes like proteases and esterases in addition to cellulases and hemicellulases, the fungi have an additional advantages of better penetration of the lignocellulosic feeds over the cellulose-degrading bacteria[85].

Bacteriophages

Bacteriophages are the viruses of bacteria and are reported to be present in the rumen in large numbers. They are specific for different bacteria present in the rumen. They are also considered to be obligate pathogens for the bacteria as bacteriophages are capable of lysing bacteria. These phages help in bacterial mass turnover in the rumen, which may be considered not so useful for the animals on different feeding schedules[86], but by lysing the bacterial cells, the bacterial protein is easily made available to the animals as a source of amino acids.

The specificity of the bacteriophages for a particular rumen bacterium may be exploited for removal or killing by lysis of unwanted rumen bacteria from the ecosystem like *Streptococcus bovis* and methanogens[87,88].

The phage population in an animal at any time is specific for that animal as the animals kept on similar diet penned together in the same shed may have diverse population of these phages. The diurnal variation in the numbers of phage particles is also very diverse. There is a drop in numbers immediately on feeding followed by a gradual increase up to 8–10 h post feeding and then decline to reach the base level[89].

Inter-relation among different groups of microbes in the rumen

Some of the rumen microbes depend upon others for the supply of nutrients required by them, while others get an-

tagonized by the excretion of anti-microbial compounds. Thus a very sensitive equilibrium must exist among these microbes for optimum fermentation in the rumen. Some of the cellulose-degrading bacteria *Ruminococcus albus* and *R. flavefaciens* produce a soluble protein that inhibits cellulose degradation by rumen anaerobic fungi[3] while some strains of chitinolytic bacteria inhibit the fungal growth[90]. *R. albus* synthesizes a bacteriocin which inhibits the growth of *R. flavefaciens*[4].

Polyplastron multivesulatum predates upon other ciliate protozoa present in the rumen like *Epidinium, Eudiplodinium, Diplodinium* and *Ostracodinium*[91]. *Entodinium bursa* has an obligate requirement for the spineless form of *E. caudatum*[92]. Protozoa in general are feeding upon rumen bacteria and play a critical role in bacterial protein turn over in the rumen. These interactions (growth promoting and antagonizing) among different microbes of the eco-system are important for maintaining a stable equilibrium which helps in optimal fermentative activity in the rumen and do not allow contaminated microbes to survive in the rumen.

Molecular techniques for rumen microbiology

As discussed above, the rumen microbial eco-system is complex and is responsible for the bioconversion of lignocellulosic feeds into volatile fatty acids. Therefore the specific role of an individual rumen microbe is important as it has to compete with the other microbes of the eco-system for its survival. The enumeration of a specific species of bacterium in the ecosystem (to quantify its role in rumen fermentation) is difficult with the conventional techniques due to a large number of biochemical tests to be performed and imprecision of the technique even for the most predominant microbe present in the ecosystem. This is perhaps due to selection pressure of the medium used for enumeration as the relative numbers of these microbes will change when cultivated in a petri dish in comparison to that metabolically active in the ecosystem. In addition, very large proportion of rumen microbes (like in any other eco-system) is non-culturable, but is active in the rumen fermentation. Therefore, it is essential to search for some better technique of quantifying specific microbes in this ecosystem, which can take care of the above drawbacks of the conventional techniques of studying microbial ecology of the rumen.

A large number of bacteria present in the rumen are non-culturable as the number of total viable bacteria on a particular medium is much lower than the microscopic count of bacteria. There is no single culture medium available which can support growth of all the culturable bacteria of rumen. Therefore, whatever information is available on the culturable bacteria of the rumen is incomplete. The classification of rumen bacteria based on phenotypic characteristics and biochemical tests is not sufficient to study the diversity among the culturable organisms. The studies on molecular biology of the known genera of rumen bacteria have shown that *Prevotella ruminicola, Butyrivibrio fibrisolvens* and *Ruminococcus* represent phylogenetically diverse groups of bacteria, in spite of the fact that the species of these genera appear to be phenotypically and biochemically similar[93]. The variation in different groups of microbes due to change of diet is difficult to study by the conventional methods of isolation and characterization of culturable microbes. Therefore, such variations in the numbers of rumen bacteria can be studied easily by using different oligonucleotide DNA probes, homologous to some regions of bacterial 16S rRNA[94]. A universal probe can be used to estimate total microbial mass and a species-specific probe can be used to determine relative proportion of total rRNA of a species of a particular bacterium. As the ratio of 16S rRNA : total cellular RNA does not change over the growth curve, 16S rRNA can be used as a measure of total bacterial population in the rumen[95]. Specific DNA probes can also be used to enumerate a single bacterial strain in a mixed population if the concentration of this particular bacterium exceeds 10 million cells per ml of the rumen contents[96].

Tajima *et al.*[97] designed and revalidated PCR primers for the detection of 13 species of rumen bacteria and these primers have been used with real-time PCR for quantification of these bacteria in the rumen microbial eco-system. This group at the University of Illinois, USA has used real-time PCR for the first time in rumen microbial ecology and appears to be one of the most important techniques to study the variations in relative microbial numbers due to change in diet and to study the establishment of an improved strain of a specific bacterium in the rumen. In addition, these primer sets can also be used for preliminary identification of new bacterial isolates from the rumen of domestic and wild animals.

These new techniques may also help in understanding the mechanism of feed utilization in the rumen. Until recently *Streptococcus bovis* was considered to be the predominant starch utilizing bacteria present in the rumen of animals fed starch-rich diet[98], but the studies with real time PCR have shown that in majority of the animals *S. bovis* numbers were not affected when the diet was shifted from forage to grain based[99] and instead a lactate-utilizing bacterium, *Megasphaera elsdenii* got established, resulting in lower lactate and higher propionate contents in the rumen. For starch hydrolysis some other microbes might be active. Therefore, it appears that with use of newer molecular biology techniques, the metabolic roles assigned to different groups of bacteria may have to be re-established and the scenario of the rumen microbiology would be entirely different in the coming decades.

Status of rumen microbiology in India

The research in rumen microbiology in India started in the seventies, but it was limited to mixed culture rumen fer-

mentation or a very few scattered studies on pure cultures of rumen microbes were carried out[30,62]. It was only in the nineties that pure culture studies were started in a few laboratories. Even now there are not more than four or five laboratories which have full facilities for the cultivation and maintenance of rumen microbes. A few bacterial and fungal cultures have been isolated from domestic (cattle and buffalo) and wild ruminants like blackbuck, chinkara, nilgai, hog deer and spotted deer[32,80] in the author's laboratory.

Why is rumen microbiology neglected in India?

- This is a low-priority area of research for microbiologists, biotechnologists and molecular biologists.
- Although it is a high-priority area for animal nutritionists, there is a lack of coordination between nutritionists and microbiologists.
- No well-established culture collection in India to provide the required microbes for research.
- Relatively difficult to cultivate and maintain rumen microbes (especially ciliate protozoa and anaerobic rumen fungi).

Future research needs

- Studies on the microbial diversity of the rumen microbiota of domestic and wild ruminants.
- Isolation/selection of rumen microbes having high micro-crystalline cellulase activity.
- Identification of microbial isolates by using new molecular biology techniques.
- Development of genetically modified microbes and their establishment in the rumen of domestic ruminants.

1. Hobson, P. N., *The Rumen Microbial Eco-system*, Elsevier Applied Science, London, 1989.
2. Thauer, R. K., Jumgermann, K. and Decker, K., Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.*, 1977, **91,** 100–180.
3. Stewart, C. S., Duncan, S. H. and Richardson, A. J., The inhibition of fungal cellulolysis by cell free preparations from ruminococci. *FEMS Microbiol. Lett.*, 1992, **97**, 83–88.
4. Odenyo, A. A., Mackie, R. I., Stahl, D. A. and White, B. A., The use of 16S rRNA probes to study competition between rumen fibrolytic bacteria: development of probe for Ruminococcus species and evidence for bacteriocin production. *Appl. Environ. Microbiol.*, 1994, **60**, 3688–3696.
5. McSweeney, C. S., Palmer, B., Krause, D. O. and Brooker, J. D., Rumen microbial ecology and physiology in sheep and goats fed a tannin containing diet. In Tannins in Livestock and Human Nutrition. Proc. International Workshop, Adelaide, Australia, 31 May – 2 June, 1999, pp. 140–145.
6. Sotohy, S. A., Sayed, A. N. and Ahmed, M. M., Effect of tannin-rich plant (*Acacia nilotica*) on some nutritional and bacteriological parameters in goats. *Deutsch.-Tierarztl. Wochenschr.*, 1997, **104**, 432–435.
7. Jones, J. A., McAllister, T. A., Muir, A. D. and Cheng, K. -J., Effects of sanfoin (*Onobrychis vicifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Appl. Environ. Microbiol.,* 1994, **60**, 1374–1378.
8. Brooker, J. D., O'Donovan, L. A., Skene, I., Clarke, K., Blackall, L. and Muslera, P. *Streptococcus caprinus* sp. nov., a tannin resistant ruminal bacterium from feral goats. *Lett. Appl. Microbiol.*, 1994, **18**, 313–318.
9. Odenyo, A. A. and Osuji, P. O., Tannin-tolerant ruminal bacteria from East African ruminants. *Can. J. Microbiol.*, 1998, **44**, 905–909.
10. Nelson, K. E., Thonney, M. L., Woolston, T. K., Zinder, S. H. and Pell, A. N., Phenotypic and phylogenetic characterization of ruminal tannin-tolerant bacteria. *Appl. Environ. Microbiol.*, 1998, **64**, 3824–3830.
11. Tjakradidjaja, A. S., Brooker, J. D. and Bottema, C. D. K., Characterization of tannin-resistant bacteria from the rumen fluid of feral goats and camels with restriction analysis of amplified 16S rDNA. In Tannins in Livestock and Human Nutrition. Proc. International Workshop, Adelaide, Australia, 31 May–2 June, 1999, pp. 151–155.
12. Sly, L. I., Cahill, M. M., Osawa, R. and Fujisawa, T., The tannin degrading species *Streptococcus gallolyticus* and *Streptococcus caprinus* are subjective synonyms. *Int. J. System. Bacteriol.*, 1997, **47**, 893–894.
13. Plumb, J. J., Blackall, L. L., Klieve, A. V. and Brooker, J. D., Rumen bacterial diversity with and without mulga (*Acacia aneura*) tannins. In Tannins in Livestock and Human Nutrition. Proc. International Workshop, Adelaide, Australia, 31 May–2 June, 1999, pp. 146–150.
14. Wiryawan, K. G., Tangendjaja, B. and Suryahadi, Brooker, J. D., Tannin degrading bacteria from Indonesian ruminants. In Tannins in Livestock and Human Nutrition. Proc. International Workshop, Adelaide, Australia, 31 May–2 June, 1999, pp. 123–126.
15. Price, K. R., Johnson, I. T. and Fenwick, G. R., The chemistry and biological significance of saponins in food and feedstuffs. *CRC Crit. Rev. Food Sci. Nutr.*, 1987, **26**, 27–135.
16. Lu, C. D., Tsai, L. S., Schaefer, D. M. and Jorgensen, N. A., Alteration of fermentation in continuous culture of mixed rumen bacteria by isolated alfalfa saponins. *J. Dairy Sci.*, 1987, **70**, 799–805.
17. Goodall, S. R., Braddy, P., Horton, D. and Beckner, B. Steam flaked vs high moisture content rations with and without sarsaponin for finishing steers. *Proc. Ann. Meet Am. Soc. Anim. Sci. West Sect.*, 1982, **33**, 45.
18. Kil, J. Y., Cho, N. K., Kim, B. S., Lee, S. R. and Maeng, W. J., Effects of yucca extract addition on *in vitro* fermentation characteristics of feed and faeces and on the milk yields in lactating cows. *Korean J. Anim. Sci.,* 1994, **36**, 698–709.
19. Wallace, R. J., Arthauo, L. and Newbold, C. J., Influence of *Yucca schidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. *Appl. Environ. Microbiol.*, 1994, **60**, 1762–1767.
20. Wang, Y., McAllister, T. A., Newbold, C. J., Rode, L. M., Cheeke, P. R. and Cheng, K. -J., Effects of *Yucca schidegera* extract on fermentation and degradation of steroidal saponins in the rumen simulation technique (RUSITEC). *Anim. Feed Sci. Technol.*, 1998, **74**, 143–153.
21. Thalib, A., Widiawati, Y., Hamid, H., Suharman, D. and Sabrani, M., The effects of saponin from *Sapindus rarak* fruit on rumen microbes and performance of sheep. *Jurnal Ilmu Ternak dan Veteriner.*, 1996, **2**, 17–21.
22. Jones, R. J. and Megarrity, R. G., Successful transfer of dihyroxypyridine-degrading bacteria from Hawaiian (USA) goats to Australian ruminants to overcome the toxicity of *Leucaena*. *Aust. Vet. J.,* 1986 **63**, 259–262.

23. Allison, M. J., Mayberry, W. R., McSweeney, C. S. and Stahl, D. A., *Synergistes jonesii,* gen. sp. nov.: A rumen bacterium that degrades toxic pyridinediols. *Syst. Appl. Microbiol.*, 1992, **15**, 522–529.

24. Hammond, A. D., Allison, M. J., Williams, G. M., Prine, G. M. and Bates, D. B., Prevention of leucaena toxicity of cattle in Florida by ruminal inoculation with 3-hydroxy-4(1H)-pyridone degrading bacteria. *Am. J. Vet. Res.*, 1989, **50**, 2176–2180.

25. McSweeney, C. S., Mackie, R. I., Odenyo, A. A. and Stahl, D. A., Development of an oligonucleotide probe targeting 16Sr RNA and its application for detection and quantification of the ruminal bacterium *Synergistes jonesii* in a mixed population chemostat. *Appl. Environ. Microbiol.*, 1993, **59**, 1607–1612.

26. Takahashi, J., Masuko, T., Endo, S., Dodo, K. and Fujita, H., Effects of dietary protein and energy levels on the reduction of nitrate and nitrite in the rumen and methemoglobin formation in sheep. *Jpn. J. Zootech. Sci.*, 1980, **51**, 626–631.

27. Allison, M. J. and Reddy, C. A., Adaptations of gastrointestinal bacteria in response to changes in dietary oxalate and nitrate. *Current Perspectives on Microbial Ecology* (eds Klug, M. J. and Reddy, C. A), Washington DC, 1984, pp. 248–256.

28. Panjarathinum, R. and Laxminarayana, H., Studies on rumen microflora in cows and buffaloes under different feeding regimes. 4. Distribution of bacteria and in vitro studies. *Indian J. Anim. Sci.,* 1977, **45**, 173–182.

29. Jalaludin, S., Ho, Y. W., Abdullah, N. and Kudo, H., Rumen microorganisms of the water buffalo. *Buffalo J.*, 1992, **8**, 211–220.

30. Sinha, R. N. and Ranganathan, B., Cellulolytic bacteria in buffalo rumen. *J. Appl. Bacteriol.*, 1983, **54**, 1–6.

31. Kumar, U., Sareen, V. K. and Singh, S., Effect of yeast culture supplement on ruminal microbial populations and metabolism in buffalo calves fed a high roughage diet. *J. Sci. Food Agric.*, 1997, **73**, 231–236.

32. Sahu, N. P., Kamra, D. N. and Paul, S. S., Effect of cellulose degrading bacteria isolated from wild and domestic ruminants on *in vitro* digestibility of feed and enzyme production. *Asian Aust. J. Anim. Sci.*, 2004, **17**, 199–202.

33. Joblin, K. N., Naylor, G. E. and Williams, A. G., Effect of *Methanobrevibacter smithii* on xylanolytic activity of anaerobic ruminal fungi. *Appl. Environ. Microbiol.*, 1990, **56**, 2287–2295.

34. Jarvis, G. N., Strompl, C., Burgess, D. M., Skillman, L. C., Moore, E. R. B. and Joblin, K. N., Isolation and identification of ruminal methanogens from grazing cattle. *Curr. Microbiol.*, 2000, **40**, 327–332.

35. Stumm, C. K., Gijzen, H. J. and Vogels, G. D., Association of methanogenic bacteria with ovine rumen ciliates, *Br. J. Nutr.*, 1982, **47**, 95–99.

36. Gruby, D. and Delafond, H. M. O., Recherches sue des animalcules se devellopant en grand nombre dans l'estomac et dans les intestines pendant la digestion des animaux herbivores et carnivores. *C. R. Hebd. Seances Acad. Sci.*, *Paris*, 1843, **17**, 1304–1308.

37. Hungate, R. E., *Rumen and its Microbes*, Academic Press, Washington DC, 1966.

38. Williams, A. G. and Coleman, G. S., *The Rumen Protozoa*, Springer Verlag, New York, 1992.

39. Kamra, D. N. and Pathak, N. N., *Nutritional Microbiology of Farm Animals,* Vikas Publishing House, New Delhi, 1996, pp. 1–224.

40. Sahu, N. P. and Kamra, D. N., Microbial eco-system of the gastro-intestinal tract of wild herbivorous animals. *J. Appl. Anim. Res.*, 2002, 21, 207–230.

41. Dehority, B. A., Protozoa of the digestive tract of herbivorous mammals. *Insect Sci. Appl.*, 1986, **7**, 279–296.

42. Eloff, A. K. and Van Hoven, W., Intestinal protozoa of the African elephant *Loxodonta africana* (Blumenbach). *South Afr. J. Zool.*, 1980, **15**, 83–90.

43. Dogiel, V. A., La faune d'infusoires inhabitant l'estomac du buffle et du dromadaire. *Ann. Parasitol.*, 1928, **6**, 323–338.

44. Thurston, J. P. and Grain, J., Holotrich ciliates from the stomach of *Hippopotamus amphibius*, with descriptions of two new genera and four new species. *J. Protozool.*, 1971, **18**, 131–141.

45. Mould, D. L. and Thomas, G. J., The enzymic degradation of starch by holotrich protozoa from sheep rumen. *Biochem. J.*, 1958, **69**, 327–337.

46. Abou Akkada, A. R. and Howard, B. H., The biochemistry of rumen protozoa. 4. Decomposition of pectic substances. *Biochem. J.*, 1961, **78,** 512–517.

47. Bailey, R. W. and Howard, B. H., The biochemistry of rumen protozoa. 6. The maltases of *Dasytricha ruminantium*, *Epidinium ecaudatum* (Crawley) and *Entodinium caudatum. Biochem. J.*, 1963, **86**, 446–452.

48. Williams, A. G., The selectivity of carbohydrate assimilation in the anaerobic rumen ciliate *Dasytricha ruminantium. J. Appl. Bacteriol.*, 1979, **47**, 511–520.

49. Williams, A. G. and Coleman, G. S., Hemicellulose degrading enzymes in rumen ciliate protozoa. *Curr. Microbiol.*, 1985, **12**, 85–90.

50. Hobson, P. N. and Stewart, C. S., *The Rumen Microbial Ecosystem*, Blackie Academic & Professional, London, 1997.

51. Punia, B. S., Microbial colonization in the rumen and utilization of ligno-cellulosic feeds. *Indian J. Microbiol.*, 1992, **32**, 235–247.

52. Sahu, N. P. and Kamra, D. N., Rumen microbial eco-system of buffalo – A review. *J. Buffalo Sci. Technol.*, 2001, **7**, 70–82.

53. Shimizu, M., Kinoshita, M., Fujita, J. and Imai, S., Rumen ciliate protozoal fauna and composition of the Zebu cattle, *Bos indicus* and water buffalo, *Bubalus bubalis* in Philippines. *Bull. Nippon Vet. Zootech. Coll.*, 1983, **32**, 83–88.

54. Dehority, B. A., Ciliate protozoa in the rumen of Brazilian water buffalo, *Bubalus bubalis* Linnaeus. *J. Protozool.*, 1979, **26**, 536–544.

55. Imai, S., Abdullah, N., Ho, H. W., Jalaludin, S., Hussain, H. Y., Onodera, R. and Kudo, H., Comparative study on the rumen ciliate populations in small experimental herds of water buffalo and Kedah Kelantan cattle in Malaysia. *Anim. Feed Sci. Technol.*, 1995, **52**, 345–351.

56. Imai, S., Han, S. S., Cheng, K. -J. and Kudo, H., Composition of the rumen ciliate population in experimental herds of cattle and sheep in Lethbridge, alberta, Western Canada. *Can. J. Microbiol.*, 1989, **35**, 686–690.

57. Dumag, P. U., Ciliates of the rumen of the Philippine carabao (*Bubalus bubalis* L.). *Philipp. J. Anim. Ind.*, 1977, **32**, 42–93.

58. Ogimoto, K., Imai, S., Asada, T. and Fujita, J., Bacterial flora, protozoal fauna and volatile fatty acids in the rumen of water buffalo (*Bubalus bubalis*) in tropical Asia. *S. Afr. J. Anim. Sci.,* 1983, **13**, 59–61.

59. Imai, S., Four new rumen ciliates, *Entodinium ogimotoi* sp. n., *E. bubalum* sp. n., and *E. tsunodai* sp. n. and *Oligoisotricha bubali* (Dogiel, 1928) n. comb. *Jap. J. Vet. Sci.*, 1981, **43**, 201–209.

60. Franzolin, R. and Dehority, B. A., Comparison of protozoal populations and digestion rates between water buffalo and cattle fed on all forage diet. *J. Appl. Anim. Res.*, 1999, **16**, 33–46.

61. Dehority, B. A., Damron, W. S. and McLaren, J. B., Occurrence of the rumen ciliate *Oligoisotricha bubali* in domestic cattle (*Bos taurus*). *Appl. Environ. Microbiol.*, 1983, **45**, 1394–1397.

62. Srivastava, R. V. N. and Chaturvedi, M. L., Studies on the rumen microbes of cattle and buffalo. *Indian J. Anim. Sci.*, 1973, **43**, 615–617.

63. Singh, U. B., Verma, D. N., Varma, A. and Ranjhan, S. K., Measurement of the rate of production of protozoa with the help of ^{35}S in the rumen of cattle and buffalo. *Indian J. Anim. Sci.*, 1974, **44**, 89–91.

64. Kurar, C. K., Gupta, B. N. and Mohini, M., Protozoal status in strained rumen liquor of cattle and buffaloes. *Indian J. Anim. Sci.*, 1988, **58**, 112–115.

65. Santra, A., Kamra, D. N. and Pathak, N. N., Influence of ciliate protozoa on biochemical changes and hydrolytic enzymes in the rumen of buffalo (*Bubalus bubalis*). *Buffalo J.*, 1995, **12**, 95–100.

66. Eadie, J. M. and Mann, S. O., Development of rumen microbial population: high starch diet and instability. In *Physiology of Digestion and Metabolism in the Ruminant* (ed. Phillipson, A. T.), Oreil Press, New Castle Upon Tyne, UK, 1970, pp. 335–347.

67. Newbold, C. J., Williams, A. G. and Chamberlain, D. G., The *in vitro* metabolism of D,L-lactic acid by rumen microorganisms. *J. Sci. Food Agric.*, 1987, **38**, 9–18.

68. Ushida, K., Jouany, J. P. and Thivend, P., Role of protozoa in nitrogen digestion in sheep given two isonitrogenous diets. *Br. J. Nutr.*, 1986, **56**, 407–419.

69. Whitelaw, F. G., Eadei, J. M., Bruce, A. L. and Shand, J. W., Ethane formation in faunated and ciliate free cattle and its relationship with rumen volatile fatty acid proportions. *Br. J. Nutr.*, 1984, **52**, 261–275.

70. Santra, A., Kamra, D. N. and Pathak, N. N., Effect of defaunation on nutrient digestibility and growth of Murrah buffalo (*Bubalus bubalis*) calves. *Int. J. Anim. Sci.*, 1994a, **9**, 185–187.

71. Santra, A., Kamra, D. N., Pathak, N. N. and Khan, M. Y., Effect of protozoa on the loss of energy in Murrah buffalo (*Bubalus bubalis*) calves. *Buffalo J.*, 1994b, **10**, 249–253.

72. Pal, D., Kamra, D. N., Pathak, N. N. and Singh, R., Effect of protozoa in the rumen and protein level in diet on nutrient utilization and performance of crossbred calves. *Int. J. Anim. Sci.*, 1994, **9**, 289–292.

73. Liebetanz, E., Die parasitischen Protozoen der Wiederkauermagens. *Arch. Prot.*, 1910, **19**, 19–90.

74. Braune, R., Untersuchungen uber die in Wiederkauermagen vorkommenden Protozoen. *Arch. Prot.*, 1913, **32**, 111–170.

75. Orpin, C. G., Studies in the rumen flagellate Neocallimastix frontalis. *J. Gen. Microbiol.*, 1975, **91**, 249–262.

76. Orpin, C. G., The occurrence of chitin in the cell walls of the rumen organisms *Neocallimastix frontalis, Piromonas communis* and *Sphaeromonas communis*. *J. Gen. Microbiol.*, 1977a, **99**, 215–218.

77. Williams, A. G. and Orpin, C. G., Glycoside hydrolase enzymes present in the zoospore and vegetative stages of the rumen fungi *Neocallimastix frontalis, Piromonas communis* and an unidentified isolate grown on a variety of carbohydrates. *Can. J. Bot.*, 1987a, **33**, 427–434.

78. Williams, A. G. and Orpin, C. G., Polysaccharide degrading enzymes formed by three species of anaerobic fungi grown on a range of carbohydrate substrates. *Can. J. Bot.*, 1987b, **33,** 418–426.

79. Hebraud, M. and Fevre, M., Characterisation of glycoside and polysaccharide hydrolases secreted by the rumen anaerobic fungi *Neocallimastix frontalis, Sphaeromonas communis* and *Piromonas communis*. *J. Gen. Microbiol.*, 1988, **134**, 1123–1129.

80. Paul, S. S., Kamra, D. N., Sastry, V. R. B., Sahu, N. P. and Kumar, A., Effect of phenolic monomers on growth and hydrolytic enzyme activities of an anaerobic fungus isolated from wild nilgai (*Boselaphus tragocamelus*). *Lett. Appl. Microbiol.*, 2003, **36**, 377–381.

81. Akin, D. E. and Rigsby, L. L., Mixed fungal populations and lignocellulosic tissues degradation in the bovine rumen. *Appl. Environ. Microbiol.,* 1987, **53**, 1987–1995.

82. Kamra, D. N., Saha, S., Bhatt, N., Chaudhary, L. C. and Agarwal, N., Effect of diet on enzyme profile, biochemical changes and *in sacco* degradability of feeds in the rumen of buffalo. *Asian Aust. J. Anim. Sci.*, 2003, **16**, 374–379.

83. Roger, V., Fonty, G., Komisarczuk, S. and Gouet, Ph., Effects of physicochemical factors on the adhesion to cellulose Avicel of the ruminal bacteria *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* sub sp. *Succinogenes*. *Appl. Environ. Microbiol.*, 1990, **56**, 3081–3087.

84. Orpin, C. G., The rumen flagellate *Piromyces communis*: Its life history and invasion of plant material in rumen. *J. Gen. Microbiol.*, 1977b, **99**, 107–117.

85. Fonty, G. and Joblin, K. N., Rumen anaerobic fungi: their role and interactions with other rumen microorganisms in relation with fibre digestion. In *Physiological Aspects of Digestion and Metabolism in Ruminants* (eds Tsuda, T., Sasaki, Y. and Kawashima, R.), Academic Press, San Diego, 1990, pp. 655–680.

86. Klieve, A. V. and Swain, R. A., Estimating ruminal bacteriophage numbers using pulsed field gel electrophoresis and laser densitometry. *Appl. Environ. Microbiol.*, 1993, **59**, 2299–2303.

87. Klieve, A. V., Heck, G. L., Prance, M. A. and Shu, Q., Genetic homogeneity and phage susceptibility of ruminal strains of *Streptococcus bovis* isolated in Australia. *Lett. Appl. Microbiol.*, 1999, **29**, 108–112.

88. Bach, S. J., Mcallister, T. A., Veira, D. M., Gannon, V. P. J. and Holley, R. A., Transmission and control of *Escherichia coli* O157:H7 – A review. *Can. J. Anim. Sci.*, 2002, **82**, 475–490.

89. Swain, R. A., Nolan, J. V. and Klieve, A. V., Natural variability and diurnal fluctuations within the bacteriophage population of the rumen. *Appl. Environ. Microbial.*, 1996, **62**, 994–997.

90. Kopecny, J., Hodrova, B. and Stewart, C. S., The effect of rumen chitinolytic bacteria on cellulolytic anaerobic fungi. *Lett. Appl. Microbiol.*, 1996, **23**, 199–202.

91. Eadie, J. M., Studies on the ecology of certain rumen ciliate protozoa. *J. Gen. Microbiol.*, 1967, **49**, 175–194.

92. Coleman, G. S., Laurie, J. I. and Bailey, J. E., The cultivation of the rumen ciliate *Entodinium bursa* in the presence of *Entodinium caudatum*. *J. Gen. Microbiol.*, 1977, **101**, 253–258.

93. Forster, R. J., Teather, R. M., Gong, J. and Deng, S. -J., 16S rDNA analysis of *Butyrivibrio fibrisolvens*: Phylogenetic position and relation to butyrate producing anaerobic bacteria from the rumen of white tailed deer. *Lett. Appl. Microbiol.*, 1996, **23**, 218–222.

94. Stahl, D. A., Flesher, B., Mansfield, H. R. and Montgomery, Use of phylogenetically based hybridization probes for studies of ruminal microbial ecology. *Appl. Environ. Microbiol.*, 1988, **54**, 1079–1084.

95. Briesacher, S. L., May, T., Grigsby, K. N., Kerley, M. S., Anthony, R. V. and Paterson, J. A., Use of DNA probe to monitor nutritional effects on ruminal 'raline'tes and *Fibrobacter succinogenes* S85. *J. Anim. Sci.,* 1992, **70**, 289–295.

96. Attwood, G. T., Lockington, R. A., Ue, G. P. and Brooker, J. D., Use of a unique gene sequence as a probe to enumerate a strain of *Bacteroides ruminicola* introduced into the rumen. *Appl. Environ. Microbiol.*, 1988, **54**, 534.

97. Tajima, K., Aminov, R. I., Nagamine, T., Matsui, H., Nakamura, M. and Benno, Y., Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. *Appl. Environ. Microbiol.*, 2001, **67**, 2766–2774.

98. Mackie, R. I. and Gilchrist, F. M. C., Changes in lactate producing and lactate utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high concentrate diet. *Appl. Environ. Microbiol.*, 1979, **38**, 422–430.

99. Klieve, A. V., Hennessy, D., Ouwerkerk, D., Forster, R. J., Mackie, R. I. and Attwood, G. T., Establishing populations of *Megasphaera elsdenii* YE 34 and *Butyrivibrio fibrisolvens* YE 44 in the rumen of cattle fed high grain diets. *J. Appl. Microbiol.*, 2003, **95**, 621–630.

100. Hungate, R. E., The anaerobic mesophilic cellulolytic bacteria. *Bacteriol. Rev.*, 1950, **14**, 1–49.

101. Flint, H. J., McPherson, C. A., Avgustin, G. and Stewart, C. S., Use of a cellulase encoding gene probe to reveal restriction fragment length polymorphisms among ruminal strains of *Bacteroides succinogenes*. *Curr. Microbiol.*, 1990, **20**, 63–68.

102. Dehority, B. A. and Scott, H. W., Extent of cellulose and hemi-cellulose digestion in various forages by pure cultures of rumen bacteria. *J. Dairy Sci.*, 1967, **50**, 1136–1141.

103. Stewart, C. S., Dinsdale, D., Cheng, K. –J. and Paniagua, C., The digestion of straw in the rumen. In *Straw Decay and its Effect on Disposal and Utilization (*ed. Grossbard, E.), Wiley, Chichester, 1979, pp. 123–130.

104. Bryant, M. P., *Ruminococcus*. In *Bergey's Manual of Systematic Bacteriology* (ed. Sneath, P. H. A.), Williams and Wilkins, Baltimore, 1986, vol. 2, pp. 1093–1097.

105. Hungate, R. E., Studies on cellulose fermentation 1. The culture and physiology of an anaerobic cellulose digesting bacterium. *J. Bacteriol.*, 1944, **48**, 499–513.

106. Hungate, R. E., Microorganisms in the rumen of cattle fed a constant ration. *Can. J. Microbiol.*, 1957, **3**, 289–311.

107. Bryant, M. P., Small, N., Bouma, C. and Robinson, I. M., Studies on the composition of the ruminal flora and fauna of young calves. *J. Dairy Sci.*, 1958, **41**, 1747–1767.

108. Van Gylswyk, N.O. and Hoffman, J. S. L., Characteristics of cellulolytic Cillobacteria from the rumens of sheep fed teff (*Eragrostis tef*) hay diets. *J. Gen. Microbiol.*, 1970, **60**, 381–386.

109. Bryant, M. P. and Burkey, L. A., Numbers and some predominant groups of bacteria in the rumen of cow fed different rations. *J. Dairy Sci.*, 1953, **36**, 218–224.

110. Bryant, M. P. and Small, N., Characteristics of two new genera of anaerobe curved rods isolated from the rumen of cattle. *J. Bacteriol.*, 1956, **72**, 22–26.

111. Cotta, M. A., Interaction of ruminal bacteria in the production and utilization of maltooligosaccharides from starch. *Appl. Environ. Microbiol.*, 1992, **58**, 48–54.

112. Van Gylswyk, N. O. and Van der Toorn, J. J. T. K., *Eubacterium uniforme* sp. nov. and *Eubacterium xylanophilum* sp. nov., fibre digesting bacteria from the rumina of sheep fed stover. *Int. J. Syst. Bacteriol.*, 1985, **35**, 323–326.

113. Latham. M. J., Sharpe, E. and Weiss, N., Anaerobic cocci from the bovine alimentary tract, the amino acids of their cell wall peptidoglycans and those of various species of anaerobic *Streptococcus. J. Appl. Bacteriol.*, 1979, **47**, 209–221.

114. Hamlin, L. J. and Hungate, R. E., Culture and physiology of a starch digesting bacterium (*Bacteroides amylophilus,* nov. sp.) from the bovine rumen. *J. Bacteriol.*, 1956, **72**, 548–554.

115. Stackebrandt, E. and Hippe, H., Transfer of *Bacteroides amylophilus* to a new genus *Ruminobacter* gen. nov. *Syst. Appl. Microbiol.*, 1986, **8**, 204–207.

116. Caldwell, D. R. and Bryant, M. P., Medium without rumen fluid for non selective enumeration and isolation of rumen bacteria. *Appl. Microbiol.*, 1966, **14,** 794–801.

117. Stewart, C. S., Lactic acid bacteria in the rumen. In *The Lactic Acid Bacteria*, Vol. 1. *The Lactic Acid Bacteria in Health and Disease* (ed. Wood, B. J. B.), Elsevier, New York, 1992, pp. 49–68.

118. Scardovi, V., The Genus *Bifidobacterium*. In *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria* (eds Starr, M. P., Stolp, H. and Truper, H. G.), Springer Verlag, Berlin, 1981, vol. 2, pp. 1951–1961.

119. Scardovi, V., Trovatelli, L. D., Crociani, F. and Sgorbati, B., Bifidobacteria in bovine rumen. New species of the genus *Bifidobacterium: B. globosum* n. sp. and *B. ruminale* n. sp. *Arch. Microbiol.*, 1969, **68**, 278–294.

120. Biavati, B. and Mattarelli, P., *Bifidobacterium ruminantium* sp. nov. and *Bifidobacterium merycicum* sp. nov. from the rumen of cattle. *Int. J. Syst. Bacteriol.*, 1991, **41**, 163–168.

121. Paster, B. J. and Canale-Parola, E., *Treponema saccharophilum* sp. nov., a large pectinolytic spirochaete from the bovine rumen. *Appl. Environ. Microbiol.*, 1985, **50**, 212–219.

122. Bryant, M. P., Barrentine, B. F. and Sykes, J. F., Predominant bacteria in the rumen of cattle on bloat provoking ladino clover pasture. *J. Dairy Sci.*, 1960, **43**, 1435–1444.

123. Hobson, P. N. and Howard, B. H., Microbial transformations. In *Handbuch der Tierernärung*, Verlag Paul Parey, Hamburg, 1969, vol. I.

124. Allison, M. J., Production of branched chain volatile fatty acids by certain anaerobic bacteria. *Appl. Environ. Microbiol.*, 1978, **35**, 872–877.

125. Wallace, R. J., Catabolism of amino acids by *Megasphaera elsdenii* LCI. *Appl. Environ. Microbiol.*, 1986, **51**, 1141–1143.

126. Elsden, S. R., Volcani, B. E., Gilchrist, F. M. C. and Lewis, D., Properties of a fatty acid forming organism from the rumen of sheep. *J. Bacteriol.*, 1956, **72**, 681–689.

127. Hobson, P. N., Mann, S. O. and Oxford, A. E., Some studies on the occurrence and properties of a large Gram-negative coccus from the rumen. *J. Gen. Microbiol.*, 1958, **19**, 462–472.

128. Wolin, M. J., Wolin, E. A. and Jacobs, N. J., Cytochrome producing anaerobiv vibrio, *Vibrio succinogenes*, sp. nov., *J. Bacteriol.*, 1961, **81**, 911–917.

129. Johns, A. T., Isolation of a bacterium producing propionic acid from the rumen of sheep. *J. Gen. Microbiol.*, 1951, **5**, 317–325.

130. Allison, M. J., Dawson, K. A., Mayberry, W. R. and Foss, J. G., *Oxalobacter formigenes* gen. nov. sp. nov.: oxalate degrading anaerobes that inhabit gastro-intestinal tract. *Arch. Microbiol.*, 1985, **141**, 1–7.

131. Bennink, M. R. and Bryant, M. P., Sulphate and nitrate reduction by a rumen strain of *Desulfovibrio desulfuricans*. In Proceedings of the 12th Biennial Conference on Rumen Function. ARS/USDA, Chicago, 1973, p. 19.

132. Howard, B. H. and Hungate, R. E., *Desulfovibrio* of the sheep rumen. *Appl. Environ. Microbiol.*, 1976, **32**, 598–602.

133. Campbell, L. L. and Singleton, R., Desulphotomaculum. In *Bergey's Manual of Systematic Bacteriology* (ed. Sneath, P. H. A.), Williams and Wilkins, Baltimore, 1986, vol. 2, pp. 1200–1202.

134. Van Gylswyk, N. O., *Succiniclasticum ruminis* gen. nov. sp. nov., a rumen bacterium converting succinate to propionate as sole energy yielding mechanism. *Int. J. Syst. Bacteriol.*, 1995, **45**, 297–300.

135. Hobson, P. N. and Mann, S. O., The isolation of glycerol fermenting and lipolytic bacteria from the rumen of sheep. *J. Gen. Microbiol.*, 1961, **25**, 227–240.

136. Sharak-Genthner, B. R., Davis, C. L. and Bryant, M. P., Features of rumen and sewage sludge strains of *Eubacterium limosum*, a methanol and H_2–CO_2 using species. *Appl. Environ. Microbiol.*, 1981, **42**, 12–19.

137. Greening, R. C. and Leedle, J. A. Z., Enrichment and isolation of *Acetitomaculum ruminis*, gen. nov., sp. nov.: acetogenic bacteria-from the bovine rumen. *Arch. Microbiol.*, 1989, **151**, 399–406.

138. Krumholz, L. R. and Bryant, M P., *Eubacterium oxidoreducens* sp. nov. requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. *Arch. Microbiol.*, 1986, **144**, 8–14.

139. Lovely, D. R., Greening, R. and Ferry, J. G., Rapidly growing rumen methanogenic organism that synthesizes coenzyme M and has a high affinity for formate. *Appl. Environ. Microbiol.*, 1984, **48**, 81–87.

140. Miller, T. L., Wolin, M. J., Zhao, H. and Bryant, M. P., Characteristics of methanogens isolated from bovine rumen. *Appl. Environ. Microbiol.*, 1986, **51**, 201–202.

141. Kandler, O. and Hippe, H., Lack of peptidoglycan in the cell walls of *Methanosarcina barkeri. Arch. Microbiol.*, 1977, **118**, 57–60.

142. Patterson, J. A. and Hespell, R. B., Trimethylamine and methylamine as growth substrates for rumen bacteria and *Methanosarcina barkeri. Curr. Microbial.*, 1979, **3**, 79–83.

143. Paynter, M. J. B. and Hungate, R. E., Characterization of the sugar sequences and the glycosidic bond arrangements of immunogenic heteroglycans. *Carbohydrate Res.*, 1968, **60**, 167–178.

144. Robinson, J. P. and Hungate, R. E., *Acholeplasma bactoclasticum* sp. nov., an anaerobic mycoplasma from the bovine rumen. *Int. J. Syst. Bacteriol.*, 1973, **23**, 171–181.

145. Robinson, I. M., Allison, M. J. and Hartman, P., *Anaeroplasma abactoclasticum* gen. nov. sp. nov., an obligately anaerobic mycoplasma from the rumen. *Int. J. Syst. Bacteriol.*, 1975, **25**, 173–181.

146. Giesecke, D., Comparative microbiology of the alimentary tract,. In *Physiology of Digestion and Metabolism in the Ruminant* (eds Philipson, A. T. *et al.*), Oriel Press, Newcastle-upon-Tyne, 1970, pp. 306–318

147. Bhatia, B. L., Protozoa: ciliophora. In *The Fauna of British India including Ceylon and Burma* (ed. Sewell, R. B. S.), Taylor and Francis, London, 1936, pp. 156–357.

148. Kamra, D. N., Sawal, R. K., Pathak, N. N., Kewalramani, N. and Agarwal, N., Diurnal variations in ciliate protozoa in the rumen of blackbuck *(Antilope cervicapra)* fed green forages. *Lett. Appl. Microbial.*, 1991, **13**, 165–167.

149. Brüggeman, J. von, Giesecke, D. and Walser-Kärst, K., Beiträge zur Wildbiologie und vergleichenden Tierphysiologie. II. Mikroorganismen in Pansen von Rothirsch (Cervus elaphus) und Reh (Capreolus capreolus). *Z. Tierphysol. Tierernähr. Futtermittelkd.*, 1967, **23**, 143–151.

150. Das Gupta, M., Preliminary observations on the protozoan fauna of the rumen of the Indian goat. *Capra hircus* linn. *Arch. Protistenkunde*, 1935, **85**, 153–172.

151. Clarke, R.T.J., Ciliates of the rumen of domestic cattle (*Bos Taurus* L.). *N. Z. J. Agric. Res.*, 1964, **7**, 248–257.

152. Asada, T., Konno, T. and Katsuki, T., Studies on the contents of the rumen in Shiba goats. 1. Comparison of the contents of the rumen between Shiba goats and Saanen goats under the same feeding conditions. *Bull. Nippon Vet. Zootech. Coll.*, 1980, **29**, 69–73.

153. Wertheim, P., A new ciliate, *Entodinium bovis* n. sp., from the stomach of *Bos taurus* L. with the revision of *Entodinium exiguum, E. nanellum, E. simplex, E. dubardi dubardi* and *E. parvum. Parasitology*, 1935, **27**, 226–230.

154. Imai, S. and Ogimoto, K., Rumen ciliate protozoal fauna and bacterial flora of the zebu cattle (*Bos indicus*) and the water buffalo (*Bubalus bubalis*) in Thailand. *Jap. J. Zootech. Sci.*, 1984, **55**, 576–583.

155. Coleman, G. S., Rumen ciliate protozoa. *Adv. Parasitol.*, 1980, **18**, 121–173.

156. Banerjee, A. K., Studies on parasitic ciliate from Indian ruminants. *Proc. Zool. Soc., Calcutta*, 1955, **8**, 87–101.

157. Becker, E. R. and Talbott, M., The protozoan fauna of the rumen and reticulum of American cattle. *Iowa State College J. Sci.*, 1927, **1**, 345–371.

158. Bush, M. and Kofoid, C. A., Ciliates from the Sierra Nevada bighorn (*Ovis canadensis sierrae* Grinnell). *Univ. California Publ. Zool.*, 1948, **53**, 237–261.

159. Abou Akkada, A. R., Bartley, E. E. and Fina, L. R., Ciliate protozoa in the rumen of the lactating cow. *J. Dairy Sci.,* 1969, **52**, 1088–1091.

160. Imai, S., Katsuno, M. and Ogimoto, K., Distribution of rumen ciliate protozoa in cattle, sheep and goat and experimental transfaunation of them. *Jap. J. Zootech. Sci.*, 1978, **49**, 494–505.

161. Imai, S., Katsuno, M. and Ogimoto, K., Type of the pattern of the rumen ciliate composition of the domestic ruminants and the predator-prey interaction of the ciliates. *Jap. J. Zootech. Sci.*, 1979, **50**, 79–87.

162. Van Hoven, W., Rumen ciliates with description of two new species from three African reedbuck species. *J. Protozool.*, 1983, **30**, 688–691.

163. Dogiel, V. A., Monographie der Familie Ophryoscolecidae. *Arch. Protistenkunde*, 1927, **59**, 1–288.

164. Orpin, C. G. and Mann, E. A., *Neocallimastix patriciarum*: new member of the Neocallimasticaceae inhabiting the sheep rumen. *Trans. Br. Mycol. Soc.*, 1986, **86**, 178–181.

165. Webb, J. and Theodorou, M. K., A rumen anaerobic fungus of the genus *Neocallimastix*: ultrastructure of the polyflagellate zoospore and young thallus. *Biosystems*, 1988, **21**, 393–401.

166. Orpin, G. C., The characterization of the rumen bacterium Eadi's oval *Magnoovum* gen. nov. *eadii* sp. nov. *Arch. Microbiol.*, 1976, **111**, 155–159.

167. Wubah, D. A. and Fuller, M. S., Studies on *Caecomyces communis*: morphology and development. *Mycologia*, 1991, **83**, 303–310.

168. Gold, J. J., Heath, I. B. and Bauchop, T., Ultrastructural description of a new chytrid genus of caecum anaerobe *Caecomyces equi* gen. nov., sp. nov., assigned to the Neocallimasticaceae. *BioSystems*, 1988, **21**, 403–415.

169. Barr, D. J. S., Kudo, H., Jackober, K. D. and Cheng, K. -J., Morphology and development of rumen fungi: *Neocallimastix* sp., *Piromyces communis, Orpinomyces bovis*, gen. nov. sp. nov., *Can. J. Bot.*, 1989, **67**, 2815–2824.

170. Breton, A., Bernalier, A. and Dusser, M., *Anaeromyces mucronatus* nov. gen, nov. sp. A new strictly anaerobic rumen fungus with polycentric thallus. *FEMS Microbiol. Lett.*, 1990, **70**, 177–182.

171. Ho, Y. W. and Bauchop, T. *Ruminomyces elegans* gen. et sp. nov. A polycentric anaerobic anaerobic rumen fungus from cattle. *Mycotaxon*, 1990, **38**, 397–405.

172. Li, J., Heath, I. B. and Bauchop, T., *Piromyces mae* and *Piromyces dumbonica*, two new species of uniflagellate anaerobic chitridiomycete fungi from the hindgut of the horse and elephant. *Can. J. Bot.*, 1990, **68**, 1021–1033.