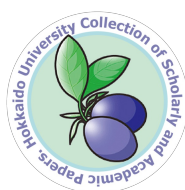




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REVIEW ARTICLE

**Inclusion of novel bacteria in rumen microbiology: needs for basic
and applied science**

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KEY WORDS: enzyme, function, microbial ecology, rumen, uncultured bacteria.

Running head: Novel bacteria in rumen microbiology

ABSTRACT

2

Rumen microbiology has made significant contributions to the understanding of ruminant
4 nutrition. However, further research progress is hindered by incomplete analysis of the
rumen microbiota comprised of bacteria, protozoa and fungi, most of which remain
6 uncharacterized due to the difficulties in isolation and cultivation. In order to maximize
rumen fiber digestion, it is necessary to understand the community structure of rumen
8 microbes, especially bacteria, and the factors that influence their composition. Recent
advances in molecular biology techniques allow the analysis of such bacteria without
10 cultivation, thereby identifying many functional, but uncultured, bacteria as new targets
for basic and applied research. Specific uncultured bacterial groups are being considered
12 as important members of a fibrolytic consortium in the rumen, judging by their ecological
distribution. Inclusion of such uncharacterized bacteria in analyses is crucial for
14 understanding the rumen microbial community and its manipulation. In addition, these
bacteria could potentially be candidates as probiotics and sources of enzymes for animal
16 feed and other industrial uses.

18 Keywords: enzyme, function, microbial ecology, rumen, uncultured bacteria.

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INTRODUCTION

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Herbivorous animals utilize plant cellulolytic materials as an energy source by
4 converting them into volatile fatty acids via fermentation of gut microbes symbiotically
acting in foregut and hindgut (Forsberg et al. 1997). In the field of animal science,
6 research over the last half-century has mainly focused on the foregut of the ruminants (the
rumen) in order to explore the physiology and ecology of symbiotic microbes. The
8 research efforts are based on maximization of animal production through optimization of
digestion and fermentation of the diet by providing suitable conditions for rumen microbes.
10 The activation and inactivation of specific microbial groups is carried out by dietary
manipulation using different feed type, feed combination, and feed additives, among other
12 methods (Krause et al. 2003; Kobayashi et al. 2004). However, in order to effectively
manipulate the fermentation, we need to appreciate the diversity and interrelationships of
14 rumen microbes in necessary for their functioning.

Wild ruminants have also been at the focus of a lot of attention regarding rumen
16 microbes by a couple of reasons. One is an academic demand for clarifying microbial
consortia, their composition and successions that are responsible for degrading a wider
18 range of fibrous materials compared to that in domestic animals. Although such analyses
are laborious, these are one of the promising approaches to the understanding the
20 mechanisms involved in anaerobic fiber digestion in the rumen. Another reason is that
wildlife under severe dietary conditions may accommodate functional microbes that are
22 not seen in domestic animals. Such microbes and their enzymes may be useful for various
industrial applications in the future.

24 Although a wealth of taxonomy, ecology and physiology data on rumen microbes, in
particular bacteria, have been accumulated, the complexity of rumen microbiota hinders
26 complete understanding of the ecosystem, as has been shown by most samples

collected/analyzed to date. The literature shows that only 10-50% of all rumen bacteria are
cultivable (Hespell et al. 1997), while the rest are totally unknown in terms of function.
The present paper reviews recent topics in rumen microbiology in domestic and wild
animals, with special reference to uncultured bacteria. The possible directions of rumen
microbiology for industrial application are also discussed.

METHODOLOGICAL ADVANCES

PCR-aided detection and quantitation

Small subunit ribosomal DNA sequence can be a powerful tool for bacterial
classification and even for tracking specific groups. Bacterial 16S rDNA libraries have
been constructed to characterize bacterial members in the rumens and also the large
intestines. Although the data obtained from such libraries are not quantitative, they are
very useful for understanding what bacterial members are present in the gut samples. One
can get a rough idea about the effects of feeds (Tajima et al. 2001), species (Daly et al.
2001), coexisting microbes (Ozutsumi et al. 2005) and digesta fractions (Larue et al. 2004)
on floral variations. Information from cloned bacterial libraries is very useful for setting
new research directions, e.g. preliminary characterization of bacterial consortia of interest
can be made by this approach (Koike et al. 2003a). The sequences accumulated to date
have been archived in large databases that are available on the free Web sites. Such
sequence information has allowed the design of probes and PCR primers specific to
certain genera, species and even strains of interest to track in defined cultures, and whole
and fractionated rumen samples. These quantitative analyses have greatly contributed to
the progress of rumen bacterial ecology.

Initially, radio-labeled DNA probes were tested to quantitate *Fibrobacter* in the rumen
(Stahl et al. 1988) and then were applied to other cellulolytic species (Weimer et al. 1999).

More sensitive competitive PCR assays were developed for several species and specific strains of rumen bacteria (Reilly & Attwood 1998; Kobayashi et al. 2000; Koike & Kobayashi 2001). At present, real-time PCR assays form the main approach to quantitating rumen bacteria with high sensitivity and reliability with minimal time requirement (Tajima et al. 2001). The advantages and disadvantages of each assay are described in the above-cited literatures. Currently, more than 15 species of rumen bacteria are assayable within 2 h once the template DNA is prepped. However, it is still crucial to know whether DNA extracted from gut samples is satisfactorily high in purity and recovery, as has been pointed out by many scientists (Yu & Morrison 2004). Also, the data should be carefully discussed, since PCR bias is always possible; in particular, when we compare real-time PCR assay values for different bacteria in different samples, this becomes an issue.

Flow cytometry and gel microdroplet

Yanagita et al. (2003) successfully separated *Oscillospira guillermontii*, a known but uncultured rumen bacterium, by a flow cytometric sorting. The separated cells were used for 16S rDNA analysis to characterize phylogeny. This is an extraordinary case, as the bacterium is distinctively large, and can be separated from other bacteria by the sorting system. However, once a specific DNA probe is developed through 16S rDNA analysis, FISH can be undertaken to separate the target bacterium with minimal damage to viability of the cells. Dyes for such FISH methods are being investigated (Manome et al. 2001). Of course, all analysis systems should be modified for use in the anaerobic chamber when the targets are anaerobic rumen bacteria.

Another potentially efficient approach to the analysis of uncultured bacteria is the use of the gel microdroplet technique, briefly presented in Figure 1, that has been introduced in the separation of uncultured bacteria living in seawater and soil (Zengler et al. 2002). In

brief, after each bacterium encapsulated in a gel microdroplet is cultivated in a synthetic medium, only gels that do not form micro-colonies (containing an uncultured bacterium) are recovered. These gels are then cultivated in a complex habitat such as soil extract that allows exchange of metabolites and signals with other indigenous bacteria. This potentially stimulates growth of the unculturable bacteria. Although this technique has not been attempted for rumen bacteria, it is worth attempting.

INSIGHTS FROM DOMESTIC ANIMALS

Cultured bacteria

There are a limited number of reports describing rumen ecology based on traditional culturing methods for separating and identifying bacteria. [Minato et al. \(1989\)](#) clarified effect of feeding ammonia-treated straw on rumen bacterial flora, showing that it stimulates growth of *Eubacterium ruminantium*. [Orpin et al. \(1985\)](#) described seasonal variation of rumen bacteria in wild reindeer by demonstrating increased cellulolytic *Butyrivibrio fibrisolvens* under severe dietary conditions in winter. Both studies were based on traditional culturing methods requiring laborious processing to obtain bacterial enumeration data.

Analysis of rumen bacteria based on 16S rDNA sequences was initiated by [Whitford et al. \(1998\)](#) followed by [Tajima et al. \(1999\)](#) using cattle rumen digesta. Since then, other libraries have been constructed and analyzed with samples from different sources (animal species and breeds, feed types, sites of gut sampling, etc.) to deepen the understanding of rumen bacterial ecology. All libraries demonstrate the presence of uncultured bacteria that will be discussed in the later sections. New aspects of culturable bacterial species obtained from the domestic ruminants are as follows.

[Koike et al. \(2003b\)](#) investigated kinetics of fiber attachment of the representative

rumen bacteria and found that *Fibrobacter succinogenes* attached to hay stems in a most rapid and extensive manner compared to ruminococci, showing maximum biomass at 24 h after stem incubation, and then is released from the stem. *Ruminococcus flavefaciens* was second most abundant, while *Ruminococcus albus* was a quite minor attaching member, indicating that contributions to fiber digestion differ among these three representative species. A similar attempt to assess the roles of cellulolytic species has clarified the importance of *F. succinogenes*. Of the 4 phylogenetically distinct groups of *F. succinogenes* (Amann et al. 1992), groups 1 through 3 were detected in the rumen of cattle and sheep (Koike et al. 2004). Our recent study indicates that the ruminal *F. succinogenes* is largely placed in the group 1, represented by the type strain S85, whether the sample is from liquid or solid fractions of rumen digesta. The group 2 is mainly distributed as a solid-associating member, while the group 3 is a quite minor group regardless the sample source (T.Shinkai et al. unpublished). Judging from these results, *F. succinogenes*, particularly those placed in the group 1, are considered to play a central role in the fiber-degrading consortium. The importance of *F. succinogenes* is indicated by many other reports (Stewart et al. 1981; Miron et al. 1989; Michalet-Doreau et al. 2001).

Interestingly, non-cellulolytic bacteria were also detected as members of fiber-associating consortia in the rumen (Koike et al. 2003a; Larue et al. 2005, Table 1). More interestingly, this depends on the fiber source (forage) used. For instance, *S. ruminantium* was frequently detected for ruminally incubated grass, such as orchardgrass and rice straw, while *Spirochaetes* including *Treponema* was often found for alfalfa and other legume plants. *S. ruminantium* is thought to utilize metabolites of *F. succinogenes*, including succinate and cellodextrins (Scheifinger & Wolin 1973) and maltodextrins (Nouaille et al. 2005). In the meantime *S. ruminantium* is considered to provide vitamin B12 and ammonia to *F. succinogenes*. Our analysis has revealed that the coexistence of *S. ruminantium* and *F. Succinogenes* increases digestibility for Avicel, orchardgrass and rice

straw by 2-5% units but not for alfalfa (Sawanon et al. 2003). Such synergistic increase of fiber digestibility was also observed for the combination of *S. ruminantium* and *R. flavefaciens* (Sawanon & Kobayashi 2006). For alfalfa, *Treponema*, another utilizer of fiber hydrolysis products, may play the same role as *S. ruminantium* does for grass. These are strongly indicative of inter-species cross feeding of some metabolites in the consortium developed for each type of plant.

Although there is no doubt that *F. succinogenes* is the primary member of the plant fiber degrading consortium, the supporting members may differ, depending on plant types (Koike et al. 2003a). Since *S. ruminantium* as well as *Treponema* is highly motile, it is interesting to know if they aid the less motile *F. succinogenes* to move together within and between plant tissues in the rumen, as this may also accelerate fiber digestion. Although competition and cooperation is observed among rumen bacteria, the analysis of various types of fibrolytic consortia may reveal more mutual activation between the bacteria. These findings are certainly of use in determining strategies for manipulating rumen microbiota and eventually rumen digestion.

Uncultured bacteria

Among rumen bacterial 16S rDNA library members, only 2-31% show a close relationship (97% or more sequence identity) with previously described species (Kobayashi 2005). Although this level of sequence identity ($\geq 97\%$) is considered to be a criterion for defining a species (Stackebrandt & Goebel 1994), the literature clearly suggests that the majority of the library constituents are unknown (unidentified) bacteria. These unknown bacteria could be classified into the following groups: viable bacteria but not identified yet, difficult to cultivate bacteria, and dead bacteria. The former two groups are targets for further characterization of ecology and physiology that will be useful for further our understanding of the rumen ecosystem and digestion.

One such unknown bacterial group has been partly characterized with the aid of the traditional nylon bag method that is usually used to determine feed digestion in the rumen. We suspended various hay stems in the rumen of sheep to characterize hay-associated bacteria, most of which are thought to contribute to the development of a bacterial consortium that facilitates fiber degradation. Hay stems withdrawn from the rumen were washed and the 16S rDNA sequences of tightly attached bacteria were retrieved to detect three groups of unknown bacteria (U1 through U3) that were placed as *Cytophaga-Flavobacter-Bacteroides* (CFB) (U1) and low GC Gram positive bacteria (LGCGP) (U2 and U3) (Koike et al. 2003a, Fig. 2).

For unknown group 2 (U2), specific real-time PCR assay and FISH were developed and used to determine the ecology in the rumen (H.Goto et al. unpublished). Members placing in U2 were distributed in the solid rather than the liquid phase of the rumen content and its time course after feeding was well synchronized with that of *F. succinogenes*, the most dominant known fibrolytic bacterial species. Phylogenetically, the known relative closest to U2 is the highly cellulolytic thermophile *Clostridium thermocellum*, suggesting that U2 may directly or indirectly contribute to fiber degradation. Furthermore, FISH analysis has revealed that U2 tightly attaches to hay stems by coexisting with other bacteria rather than existing alone. This strongly indicates that bacteria placing in U2 participate in the development of a fiber-digesting bacterial consortium on plant fragment that is not released even after the washing.

Larue et al. (2005) demonstrated the importance of fiber-associated uncultured groups belonging to a clostridium phylum that were characterized by analysis of ribosomal intergenic spacer region. Although these are not placed in U2, the lineages related to clostridia clearly require further study. Bacteria of U3, including hemicellulolytic rumen bacterium *Eubacterium ruminantium* as the closest relative, were suggested to play a minor contributory role to fiber digestion according to their ecological distribution. In fact,

more bacteria of U3 were present in the liquid rather than the solid fractions of rumen content (H.Goto et al., unpublished). Other unknown groups designated as α and β (Table 1) are presently being characterized.

Ozutsumi et al. (2005; 2006) indicated that defaunated cattle had a particular group of bacteria (CUR-E clusters) related to *Clostridium leptum*, one of the predominant groups in the human gut (Matsuki et al. 2004). The CUR-E increases its population size when protozoa are removed from the rumen. This unknown group also needs to be characterized regarding function in the rumen, especially in the protozoa-free rumen.

Another target for rumen microbiology is a previously known, but uncultured bacterium. *Oscillospira* species, a morphologically distinctive but uncultured bacteria, were partially characterized by flow cytometry sorting followed by analysis of 16S rDNA sequences (Yanagita et al. 2003) and by designing specific probes for FISH detection (Mackie et al. 2003). The research suggested that they were not involved in digestion of low quality fiber but in digestion of fresh forage.

The preceding culture-independent approaches, such as gene sequencing, quantitative PCR and FISH, certainly provide more detailed information of rumen microbial digestion in a quick, sensitive and accurate manner. In addition, the newly developed culture-dependent techniques utilizing gel microdroplet and flow cytometry sorting might lead to a breakthrough, thus promoting the comprehensive understanding of the rumen ecosystem by allowing the uncultured bacteria to be cultured.

INSIGHTS FROM WILDLIFE

Cultured bacteria

Adaptation to severe dietary conditions is one of the important strategies for the survival of wildlife. In fact, seasonal changes of dietary condition in wildlife are much

more drastic than those in domestic animals, as has been indicated in Svalbard reindeer
(Orpin et al. 1985). The cellulolytic *Butyrivibrio fibrisolvens* becomes predominant (>30%
of total isolates) in the rumen of reindeer in winter. Such a surprising increase has never
been reported for domestic ruminants. Thus, wild ruminants may have a greater ability to
tolerate low quality diets as a result of superior rumen bacterial function.

Our research team has been involved in studies on seasonal changes in diet and rumen
bacteria of wild sika deer living on the Shiretoko peninsula of Hokkaido Island, Japan,
which was registered as a UNESCO world natural heritage site. Deer diets greatly change
seasonally from fresh graminoids (mainly grass) in summer to bark and wooden fiber in
winter (Ichimura et al. 2004). Analysis of bacterial 16S rDNA clone libraries shows that
only 4% of the clones comprises of known bacterial species sharing 97% or more
sequence identity. Most of the remaining were unknown bacteria belonging to a CFB
group. This was quite different from the case of domestic ruminants having a LGCGP
group as a main phylum (Table 2). Since ruminal levels of *F. succinogenes* in the deer was
not as high as those in domestic ruminants, an alternative core member of the fibrolytic
consortium may be present in deer rumen. Three bacterial groups notably increased in
abundance under the severe dietary conditions of winter: *Streptococcus bovis*,
Ruminococcus flavefaciens, and a tentatively designated unknown group A (Yamano et al.
2003). *S. bovis* is frequently found in the rumen of domestic animals on grain-rich diets.
This discrepancy can finally be explained by the following genetic and functional
diversity of *S. bovis* and its related species.

According to 16S rDNA sequence, *S. bovis* is not perfectly distinguishable from
Streptococcus gallolyticus which is known as a tannase (tannin-degrading enzyme)
producer isolated from various animals and foods. Based on the hypothesis that most of
the bacteria quantitated as *S. bovis* in winter deer rumen are tannin-degrading *S.*
gallolyticus, we attempted to isolate tannin-degrading bacteria from the deer rumen. To

date, a total 71 tannase-producing bacteria have been successfully isolated from winter
2 deer rumen samples, while none have been isolated from samples taken in other seasons.
The sequence data of 16S rDNA and *sodA* (super oxide demutase A) confirmed that most
4 of the tannase-producing bacteria are *S. gallolyticus*, confirming the above hypothesis.
Some of these strains are more active against tannic acid compared to other
6 tannase-producing bacteria from guts of koalas and other animals (Nishitani & Osawa
2003), showing 2-3 times higher activity (Y.Sawabe et al. unpublished). Since tannin
8 forms indigestible complex with sugars and proteins, the above tannin-degrading bacteria
in deer rumen may contribute to clearance of the negative nutritional factor, tannin, from
10 the rumen to enhance digestion of fiber and conservation of protein.

Tannin-degrading bacteria have also been isolated from African ruminants; one of the
12 potent isolates from an antelope (a bush duiker) was identified as *Selenomonas*
ruminantium (Odenyo & Osuji 1998) that is being further characterized for introduction
14 into domestic ruminants to improve tolerance to tanniferous plants. It is supposed that
wild browsing ruminants such as deer and antelope accommodate tannin-degrading
16 bacteria to a higher extent compared to domestic ruminants.

18 **Uncultured bacteria**

According to comparative sequencing analysis of bacterial 16S rDNA libraries
20 constructed from rumen content of wild ruminants, the proportion of unknown bacteria is
quite high, ranging from 96% for Hokkaido sika deer (Yamano et al. 2003) to 100% for
22 Svalbard reindeer (Sundset et al. 2005) (Table 2). Semi-wild yak have the same trend,
showing a higher ratio of uncultured bacteria than the native Jinnan cattle living in the
24 surrounding areas (An et al. 2005). Accordingly, rumen samples of these wild and
semi-wild ruminants might contain novel bacteria to a greater extent, compared with those
26 of the domestic ruminants.

The uncultured rumen bacteria in wild ruminants have not yet been functionally evaluated. Of these bacteria, the unknown bacterial group A from sika deer of Shiretoko area has been partially described by Yamano et al. (2003). This group is being examined further with regard to its distribution in other ruminants, localization in rumen content, and morphology by real-time PCR and FISH analyses. A portion of the ongoing analyses suggests that the bacteria of the unknown group A are distributed in the rumens of not only deer but also sheep and cattle. In particular, this unknown group was present at a higher level in solid than in liquid fraction of rumen digesta. Although culturing of this group was thought to be difficult in initial attempts, this unknown group has been found to grow well in the presence of other bacteria in a medium containing rumen fluid from deer but not from sheep and cattle. Powdered bark of Japanese oak was the best carbon source for accumulating the bacteria of unknown group A *in vitro*. This monitoring was made possible by a newly developed group specific real-time PCR assay and FISH detection (H.Yamano et al., unpublished). More recently, a cultured bacterium was newly deposited in Genbank that has been assigned to this unknown group A (Y.Hannda et al. unpublished). Consequently, the unknown bacterial group A is now considered to include cultivable, but yet unidentified rumen bacteria.

Overall, wild ruminants may be a good source of novel bacteria, some of which might be responsible for the tolerance of these animals to low-quality diets containing less digestible fiber and more anti-nutritional factors such as tannins. Greater efforts should be made to separate and screen functional bacteria that can be used as probiotics and enzyme sources.

OPPORTUNITIES FOR APPLICATION

Culture-independent methods have made great contributions to advances in rumen

studies, most of which are basic science advances in microbial ecology. For the animal nutritionist, as well as a rumen microbiologist, these advances should be beneficial in making progress in the field of animal production. One of our research directions toward the effective use of rumen microbial data is to analyze certain microbial consortia responsible for particular functions in the rumen, *e.g.* fiber-attaching fibrolytic consortium. In this result, the fibrolytic consortia developed on various forage materials are being explored (H.Yabuki et al. unpublished). As described earlier, *F. succinogenes* was found to be necessary for development of the fibrolytic consortium as a core member and several non-cellylolytic species were considered to mainly support the activity of *F. succinogenes*. By elucidating essential members and the proportions suitable for expressing maximum activity of the consortium, “a set of bacteria” can be artificially constructed. This set could then be distributed to farmer’s level as a premix of the fibrolytic bacterial consortium that may enable safe early weaning and also abrupt changes of diet.

The idea that rumen microbes and their enzymes could be applied to industrial uses has long been proposed. As one such attempt, a phytase from rumen bacteria has been actively explored in Canada (Yanke et al. 1998). A potent phytase from *S. ruminantium* was characterized and prepared for use in feed manufacturing industry (Hong et al. 2004). Another example is a cellulose-binding module of endoglucanase from *R.albus* (Karita et al. 1996) that is being used as a tag for protein purification processes under the collaboration of a bio-industrial company. A novel cellulose-binding module has also been isolated from *Eubacterium cellulosolvens* (Yoda et al. 2006).

A cellobiose-epimerase and its gene have been isolated from *R.albus* and characterized (Matsui et al. 2005). Although the presence of this enzyme was pointed out 40 years ago (Tyler & Leatherwood 1967), no scientist has successfully analyzed it in detail. This enzyme is capable of converting cellobiose into glucosyl-mannnose and *vice versa*. Using this enzyme, various oligosaccharides can be produced more efficiently and

much cheaper than is currently possible. Screening of ruminococcal strains and other
2 ruminal strains showing high activity for this reaction is in progress by considering future
application to the food industry.

4 Attention has also been paid to bacteria themselves, *e.g.* *F. succinogenes* is
highlighted as a potent bacterium for biodegradation of lignocellulosic waste in anaerobic
6 biogas reactor (Lissens et al. 2004). Thus, including functionally unknown organisms,
symbiotic microbes in the rumen may have broad functions within and beyond animal
8 industry, as illustrated in Figure 3. Efforts should be made to further explore useful
functions among these microbes, because the herbivore gut is considered to be a treasure
10 chamber of microbes and enzymes capable of degrading and converting plant
polysaccharide that is the world's richest renewable biomass.

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Figure legends

2

Fig. 1 Use of gel microdroplet technique for separation and enrichment of uncultured
4 bacteria.

Step 1: Encapsulation of each bacterium in a gel droplet.

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Step 2: Cultivation without other bacteria in a synthetic medium.

Step 3: Elimination of gel droplets showing bacterial growth.

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Step 4: Selection of gel droplets without bacterial growth.

Step 5: Cultivation with other bacteria in a complex medium to allow exchanges of
10 metabolites and signals.

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Fig. 2 Phylogenetic placement of unknown bacterial groups U2 and U3 belonging to low
14 GC Gram positive bacteria (Koike et al. 2003a).

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Fig. 3 Possible directions for application of rumen bacterial functions.

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和文抄録

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ルーメン微生物学における研究対象としての新規細菌

4 ー基礎および応用科学からのニーズー

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ルーメン微生物学は反芻家畜栄養学の進展に多大な貢献をしてきたが、ルーメン
10 に生息する微生物（プロトゾア、真菌および細菌）の多くが難培養性であること
から個々の機能評価が難しく、更なる研究の進展がはばまれている。ルーメン内
12 繊維質消化を最大化するには、ルーメン微生物、とりわけ細菌の集団構造を理解
し、それら生態系に影響する要因を明らかにする必要がある。最近の分子生物学
14 的手法は、培養を介さない微生物生態系の解析を可能にし、それにより多くの機
能性未培養細菌の存在を明らかにした。これら未培養細菌は、実際、基礎・応用
16 双方の領域において研究対象となりつつある。未培養細菌群のいくつかは、生態
情報から推察すると、ルーメンでの繊維分解者集団の中心的な構成者であること
18 がわかってきている。このような新規の機能性細菌を考慮に入れることが、ルー
メン生態系の理解や制御には必須と思われる。さらにこれら機能性細菌や保有酵
20 素は、畜産および他の産業への応用に資するものである。

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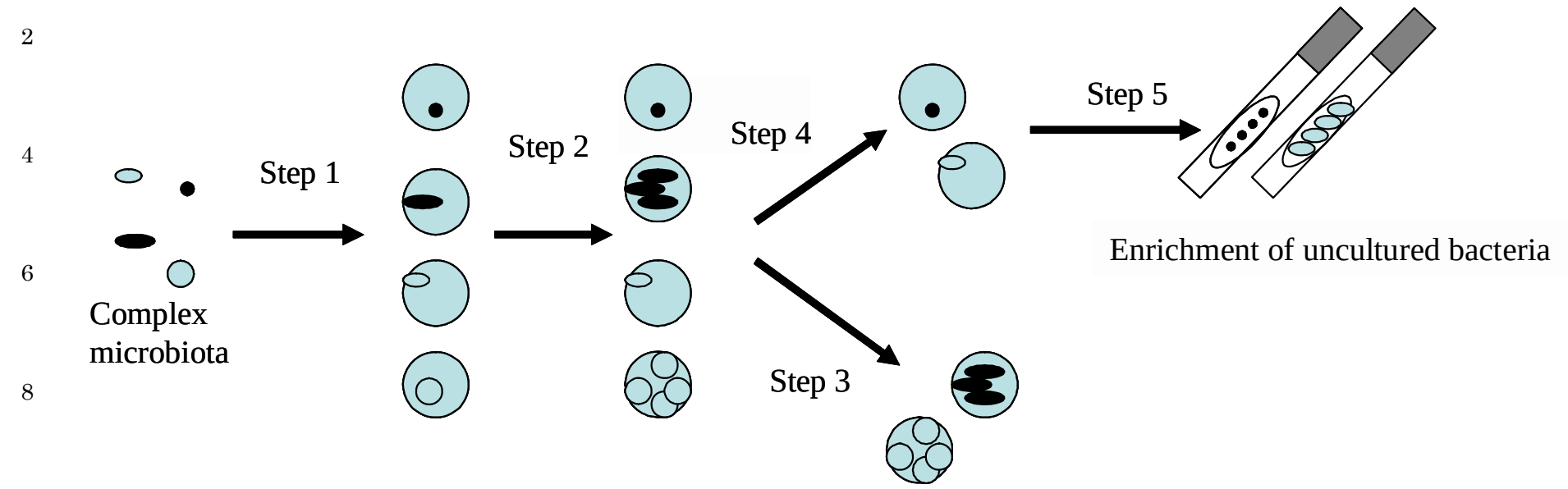


Fig.1

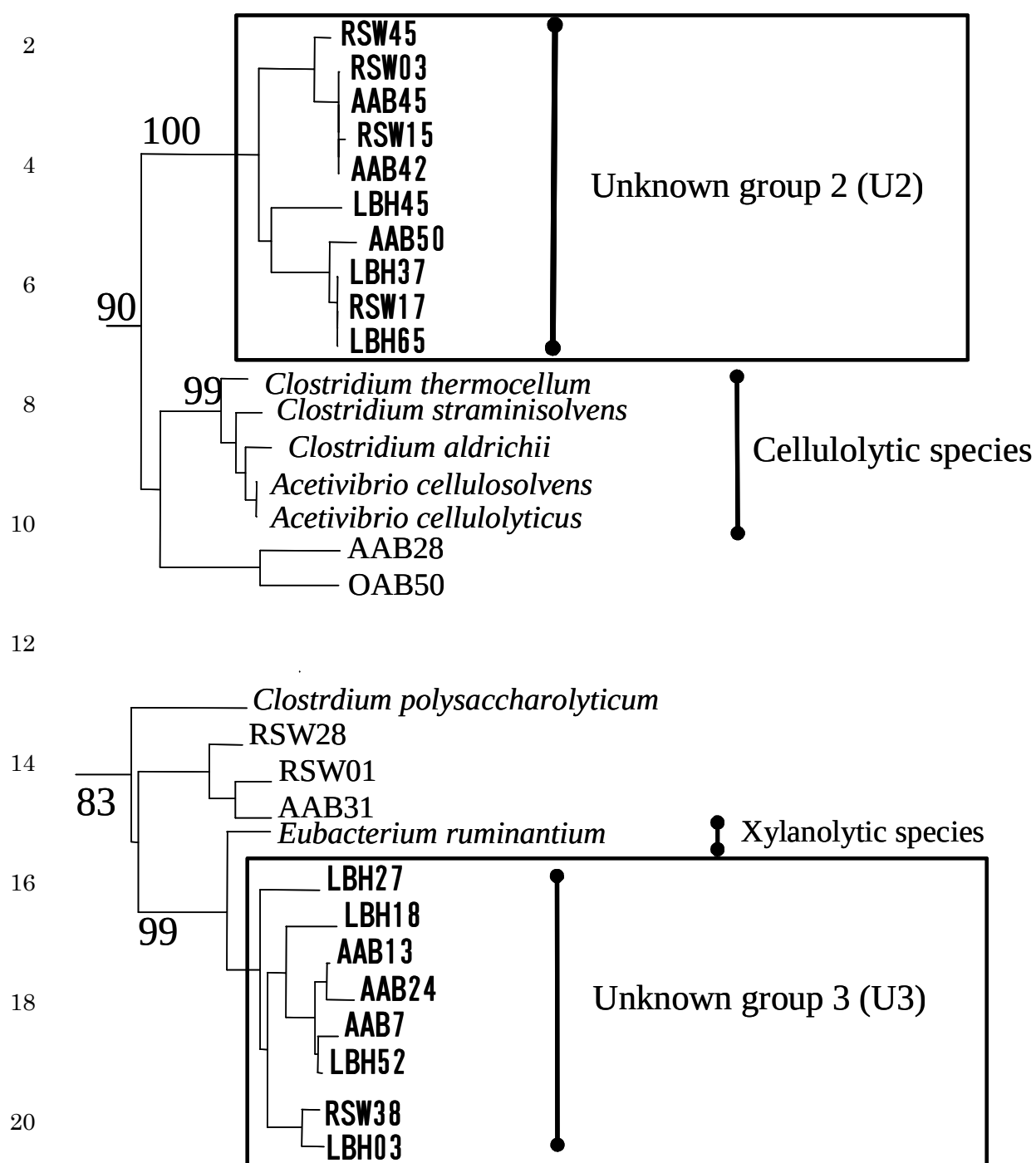


Fig.2

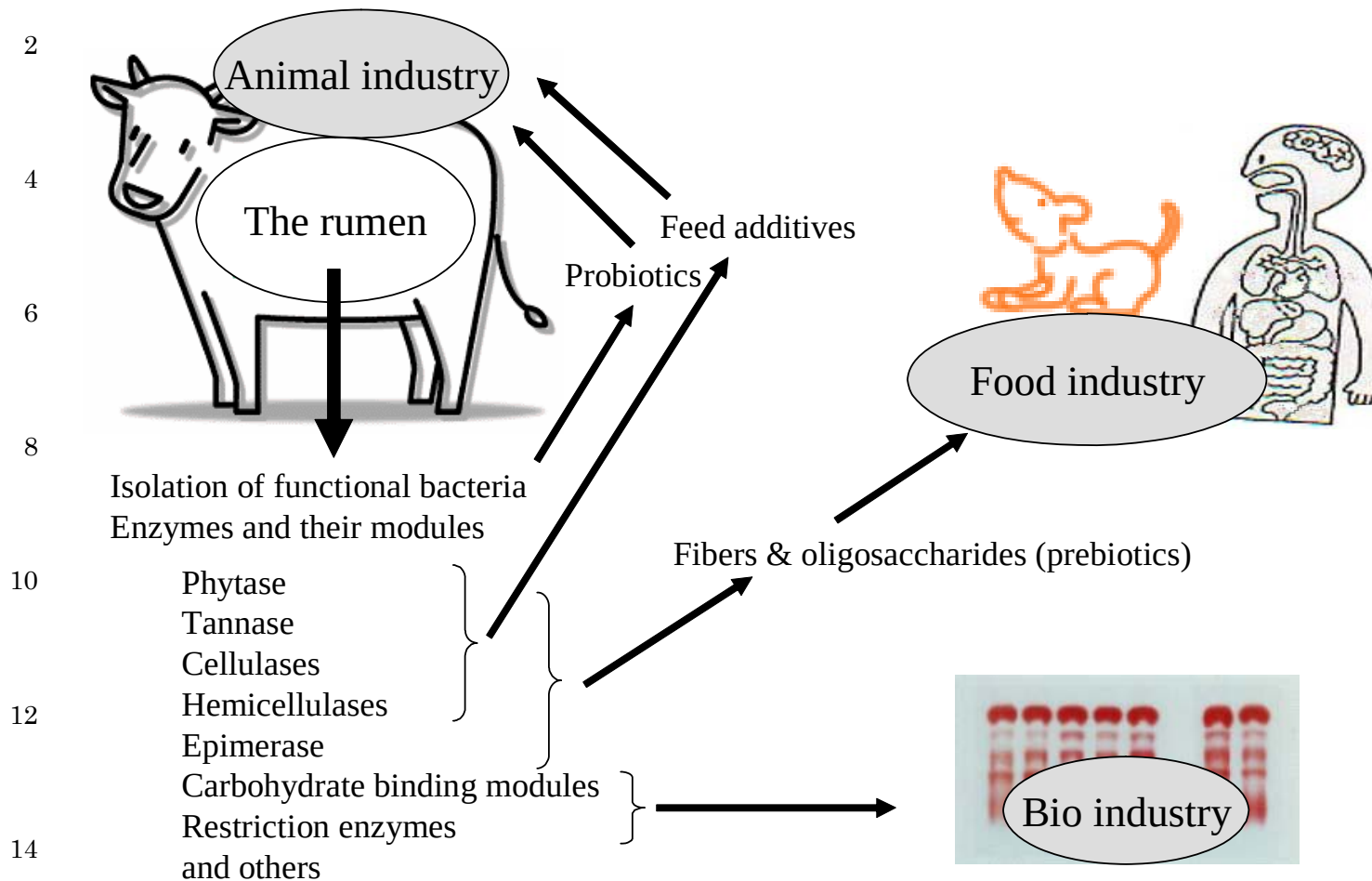


Fig.3

Table 1. Known and unknown bacteria retrieved from plant materials in the rumen that are considered as members of a fibrolytic consortium

Sources of rumen sample	Total clone number	Known bacteria*											Unknown bacteria					Known:Unknownr	Literatures
		Fs	Bf	Sr	Pr	Tb	Rf	Ra	Sd	Psr	Ss	Ca	U2	U3	□	□	others		
Ruminally incubated grass (in situ)																			
Orchard grass stem	48	1	8	2	1				1	1	2				1	31	33:67	Koike et al. (2003a)	
Rice straw stem	62	2			3							4	1	1	9	42	8:92	Fuma et al. unpublished	
Ruminally incubated legume (in situ)																			
Alfalfa stem	44	1	4									3	3	2		31	11:89	Koike et al. (2003a)	
Legume A	71	1	7		1			2				3	4	14	1	38	15:85	Fuma et al. unpublished	
Legume B	31	2	2					1						2		24	16:84	Fuma et al. unpublished	
Rumen digesta (in vivo)																			
Solid (grass & legume hay/conc fed)	46		4		1	1						4				36	13:87	Tajima et al. (1999)	
Solid (only grass hay fed)	89			1	4		1	1			1	NA	NA	NA	NA	81	9:91	Larue et al. (2005)	

*: based on >97% sequence identity in 16S rDNA except Larue et al. (2005) with >95% sequence identity in ribosomal intergenic spacer region.

Known bacteria:

Fs: *Fibrobacter succinogenes*

Bf: *Butyrivibrio fibrisolvens*

Sr: *Selenomonas ruminantium*

Pr: *Prevotella ruminicola*

Tb: *Treponema bryantii*

Rf: *Ruminococcus flavefaciens*

Ra: *Ruminococcus albus*

Sd: *Succinivibrio dextrinosolvens*

Psr *Pseudobutyrvibrio ruminis*

Ss: *Schwartzia succinovorans*

Ca: *Clostridium aminophilus*

Unknown bacteria:

U2: Unknown group 2 (Koike et al. 2003a)

U3: Unknown group 3 (Koike et al. 2003a)

□¶ Unknown group □ (Fuma et al. unpublished)

□¶ Unknown group □ (Fuma et al. unpublished)

NA: Data not available

Table 2 Rumen bacterial community of domestic and wild ruminants revealed by analysis of 16S rRNA gene library

Source	Proportion of bacteria grouped in different similarity level(%)		*Proportion of LGCGP:CFB:Others	Diets	Literatures
	>97%	97%>			
Domestic ruminants					
Cow (rumen digesta)					
Liquid	2	95	48:38:14	hay/conc	Tajima et al. (1999)
Solid	10	90	69:29:02	hay/conc	
Steer (rumen fluid)					
faunated	3	97	60:34:06	hay/conc	Ozutsumi et al. (2005)
defaunated	5	95	66:31:03	hay/conc	
Wild ruminants					
Sika deer (rumen digesta)			35:62:03		Yamano et al. (2003)
in summer	4	96	32:65:03	grass, forb etc.	
in winter	3	97	38:59:03	burk, twig etc.	
Reindeer (rumen digesta)					
on Svalbard range	0	100	55:42:03	lichen, herb, moss etc.	Sundset et al. (2005)
on Norwegian range	NA	NA	68:32:00	grass	
on artificial diet	NA	NA	91:07:02	concentrate	

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