RESEARCH ARTICLE

Rumen microbial diversity in Svalbard reindeer, with particular emphasis on methanogenic archaea

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Abstract

Ruminal methanogens, bacteria and ciliate protozoa of Svalbard reindeer grazing natural pastures in October (late fall) and April (late winter) were investigated using molecular-based approaches. The appetite of the Svalbard reindeer peaks in August (summer) and is at its lowest in March (winter). Microbial numbers, quantified by real-time PCR, did not change significantly between October and April, when food intakes are at similar levels, although the numbers of methanogens tended to be higher in October ($P = 0.074$), and ciliate numbers tended to be higher in April ($P = 0.055$). Similarly, no change was detected in the bacterial and protozoal population composition by rRNA gene-based denaturing gradient gel electrophoresis analysis. Dominant methanogens were identified using a 16S rRNA gene library (97 clones) prepared from pooled PCR products from reindeer on October pasture ($n = 5$). Eleven of the 22 distinct operational taxonomic units (OTUs) generated exhibited a high degree of sequence similarity to methanogens affiliated with *Methanobacteriales* (eight OTUs), *Methanomicrobiales* (one OTU) and *Methanosarcinales* (two OTUs). The remaining 11 OTUs (53% of the clones) were associated with a cluster of uncultivated ruminal archaea. This study has provided important insights into the rumen microbiome of a high-arctic herbivorous animal living under harsh nutritional conditions, and evidence suggesting that host type affects the population size of ruminal methanogens.

Introduction

Svalbard reindeer (*Rangifer tarandus platyrhynchus*) survive food scarcity and periods of starvation during 8 months of winter in the high-arctic desert island Svalbard (74–81°N, 10–35°E) (Fig. 1). They experience extreme variations in day length throughout the year, resulting in large seasonal changes in appetite that phase with their activity cycle (van Oort *et al.*, 2007). Maximum food intake is in August, when the food nutrient content, digestibility and availability are at its peak, with the minimum intake in March being only about one-third of that during the summer, while intake and activity are similar in October (late fall) and April (late winter) (Larsen *et al.*, 1985; Mathiesen *et al.*, 2005; van Oort *et al.*, 2007). Hence, pasture quality is high in summer, while ruminal production of energy-rich short-chain fatty acids (SCFA) is almost negligible in winter, reflecting the poor quality of their diet during this time of the year (Sørmo *et al.*, 1999; Mathiesen *et al.*, 2005).

Optimization of energy intake is essential during winter, when pasture vegetation consists of mosses and low-quality vascular plants. In contrast, during the summer, Svalbard reindeer feed on lush tundra vegetation consisting of grasses, herbs, dwarf shrubs and sedges (Tyler & Øritsland, 1989; Sørmo *et al.*, 1999; Mathiesen *et al.*, 2005). Svalbard reindeer are foregut fermenters whose digestion of dietary material depends on a symbiotic association with the complex microbiota resident within their rumen (Mathiesen *et al.*, 2005). Recent data suggest that the coevolution between reindeer and their heterogeneous diet of arctic plants has

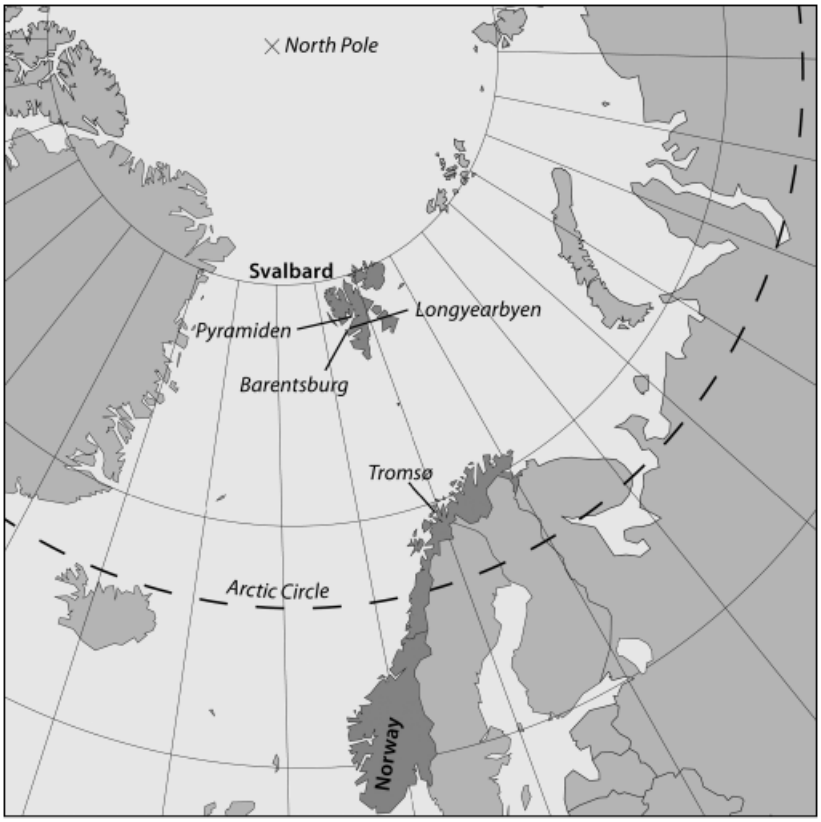

Fig. 1. Map of Svalbard and Norway. The Svalbard reindeer investigated were slaughtered grazing on natural pastures in Colesdalen and SG dalen outside Longyearbyen in Nordensköld Land, central Spitsbergen.

resulted in a unique rumen microbial ecosystem within these animals (Sundset *et al.*, 2007). In fact, $>$ 90% of the bacterial 16S rRNA genes sequenced from the reindeer rumen represented novel strains (Sundset *et al.*, 2007).

Enteric methane emission from ruminants represents not only a loss of energy to the individual animal but also a source of this greenhouse gas (Thorpe, 2008). Up to 12% of the energy intake in domestic ruminants is lost in the form of methane, a gas produced by rumen methanogenic archaea using H_2 to reduce CO_2 (Johnson & Johnson, 1995). Cultivation-based studies of ruminal methanogens in Svalbard reindeer indicate very low population densities (10^4–10^7 cells mL^{-1} rumen fluid) (Orpin *et al.*, 1985) compared with those found in domestic ruminants (10^9 cells mL^{-1} rumen fluid) (Skillman *et al.*, 2004). Low numbers of ruminal methanogens imply reduced methane emissions, something that could facilitate energy in favor of the growth and survival of these animals living under such austere nutritional conditions. Svalbard reindeer may therefore represent an interesting model to improve our understanding of the ecological importance of rumen methanogens. New insight would have practical applicability in developing strategies for reducing methane emissions from animal husbandry, as inhibition or removal of these microorganisms from the gastrointestinal tract ecosystem is considered important to reduce future climate change.

At present, knowledge of the rumen microbial ecology of Svalbard reindeer is limited to earlier cultivation-based studies (Mathiesen *et al.*, 2005), which are known to be limited in their ability to detect microorganisms compared with molecular-based approaches (Sundset *et al.*, 2007). Therefore, the objectives of the present study were to: (a) enumerate the rumen methanogens in Svalbard reindeer and assess their relative abundance compared with bacteria and protozoa using quantitative real-time PCR; (b) assess the population structure of bacteria and protozoa based on partial SSU rRNA gene analyses using denaturing gradient gel electrophoresis (DGGE); and (c) determine the phylogeny of the main ruminal methanogens present in Svalbard reindeer by constructing a 16S rRNA gene library.

Materials and methods

Animals and sampling

Five adult female Svalbard reindeer (SR1–SR5) grazing on late fall pasture (Colesdalen, near Longyearbyen, Svalbard, October 2001) and five adult females (SR6–SR10) grazing on late winter pasture (SG dalen, near Longyearbyen, Svalbard, April 2002) were sacrificed in accordance with Norwegian regulations (sections 9 and 10 in the

Norwegian Animal Welfare Act) (Fig. 1). Whole rumen contents were collected from all animals immediately after slaughter and 5-mL aliquots were immediately frozen at −20 °C for DNA extraction and molecular analysis. Subsamples of the frozen whole rumen contents were later fixed in a final concentration of 70% ethanol and stored at room temperature or at −20 °C until the commencement of real-time PCR and profiling of rumen microbial communities.

DNA extraction

The quantitative real-time PCR and DGGE profiling were performed on DNA extracted using a glass–milk extraction method as described previously (Sundset *et al.*, 2009). For the methanogenic archaea clone library, total genomic DNA was extracted using a Power Soil™ DNA isolation kit (MO BIO Laboratories Inc., Carlsbad, CA) following the manufacturer's protocol.

Quantitative real-time PCR analysis

For each microbial group (bacteria, methanogenic archaea and ciliate protozoa), three different sample aliquots of the Svalbard reindeer rumen contents were analyzed using real-time PCR as described previously by Sundset *et al.* (2009) using the bacterial 16S rRNA gene primers 1114F and 1275R (Denman & McSweeney, 2006), qmcrA-F and qmcrA-R primers targeting the methyl-coenzyme M reductase subunit for methanogenic archaea (Denman *et al.*, 2007), and P-SSU-316f and P-SSU-539r targeting the 18S rRNA gene of ciliate protozoa (Sylvester *et al.*, 2004). The external standards used for the real-time PCR amplifications have been validated previously for bacteria (Denman & McSweeney, 2006), ciliate protozoa (Sylvester *et al.*, 2004) and methanogenic archaea (Denman *et al.*, 2007). An unpaired two-tailed *t*-test was used to assess whether there was a significant effect ($P < 0.05$) of season on the quantified number of cells within individual microbial groups.

Bacterial 16S rRNA gene PCR for DGGE analysis

Amplification of the V6-8 region of the bacterial 16S rRNA gene was carried out with the primer pair F968GC and R1401 (Zoetendal & Akkermans, 1998). PCR amplifications were performed using a 2720 thermal cycler (Applied Biosystems, Warrington, UK) in 50-μL volumes containing 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 3 mM $MgCl_2$, 800 μM dNTP, 25 pmol of each primer, 1.25 U of iTaq DNA polymerase (Bio-Rad UK Ltd, Hemel Hempstead, UK) and 1.0 μL of template DNA (10 ng). Amplification conditions were: an initial denaturation of 95 °C for 3 min, followed by 35 cycles (95 °C 30 s, 56 °C 30 s and 72 °C 1 min) and a final extension of 72 °C for 5 min. After PCR, amplification of products was verified by agarose gel electrophoresis.

Ciliate protozoal 18S rRNA gene PCR for DGGE analysis

Amplification of the 18S rRNA gene of ciliate protozoa was carried out using the primers 316F (5′-GCT TTC GWT GGT AGT GTA TT-3′) and 539R (5′-ACT TGC CCT CAA ATC GT-3′, with a 40 bp GC clamp at the 5′ end) (Huws *et al.*, 2009). PCR amplifications were performed using a 2720 thermal cycler (Applied Biosystems) in 50-μL volumes containing 1 × PCR buffer (40 mM Tricine-KOH, 16 mM KCl, 3.5 mM $MgCl_2$, 3.75 μg mL^{-1} bovine serum albumin), 800 μM dNTP, 25 pmol of each primer, 1.0 μL of 50 × Titanium Taq DNA polymerase (Clontech) and 1.0 μL of template DNA (10 ng). Amplification conditions for the primer pair were: an initial denaturation of 95 °C for 3 min, followed by 35 cycles (95 °C 1 min, 56 °C 30 s and 72 °C 1 min) and a final extension of 72 °C for 10 min. After PCR, amplification of products was verified by agarose gel electrophoresis.

DGGE and gel analysis

For bacterial DGGE, samples were loaded onto a 6% (w/v) polyacrylamide gel with a 35–60% denaturing gradient [100% denaturant consisting of 40% (v/v) deionized formamide and 7 M urea]. For protozoal DGGE, samples were loaded onto an 8% (w/v) polyacrylamide gel with a 40–50% denaturing gradient. Electrophoresis was performed in a D-Code system (Bio-Rad UK Ltd). Both bacterial and protozoal DGGE gels were run initially for 10 min at 200 V, before electrophoresis for 16 h at 85 V in 0.5 × Tris-acetate-EDTA buffer (20 mM Tris-acetate, 0.5 mM EDTA) at a constant temperature of 60 °C (Heilig *et al.*, 2002). At the completion of electrophoresis, the gels were then stained with silver nitrate (Sanguinetti *et al.*, 1994) and scanned using a GS-800 calibrated imaging densitometer (Bio-Rad UK Ltd). The saved images were then imported into the software package FINGERPRINTING (Bio-Rad UK Ltd) for analysis (Fingerprint Types and Cluster Analysis modules). Cluster analysis was performed using the Dice similarity coefficient with a position tolerance of 0.5% and an optimization parameter of 1%, with clusters constructed using the unweighted pair-wise grouping with mathematical averages method. Differences in sample similarity values were considered to be significant when they were above the threshold values of 100% and 99%, respectively, for the bacteria and ciliate protozoa. Threshold values were generated by comparing the similarity of the marker lanes that were replicated in three different locations across the gel.

Methanogen 16S rRNA gene clone library

Methanogenic archaeal 16S rRNA genes were PCR amplified using the primers Met86F and Met1340R (Wright & Pimm, 2003) as described by Sundset *et al.* (2009). The 16S rRNA gene amplicons (approximately 1.2 kb) from each of the five

Svalbard reindeer on late summer pasture (SR1–SR5) were pooled and then cloned using the TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Clones containing the correct insert size were then sequenced on an ABI 3130x1 Genetic analyzer (Applied Biosystems, Foster City, CA) using ABI BigDye terminator chemistry.

Sequence analysis

Archaeal 16S rRNA gene sequences were assembled using the program LASERGENE™ SEQMAN v. 7.1.0. (DNASTAR Inc.), and then evaluated using the chimeric detection program BELLEROPHON (Huber *et al.*, 2004). Sequences were then compared with those deposited in GenBank using BASIC LOCAL ALIGNMENT SEARCH TOOL (BLAST) (Altschul *et al.*, 1997), and automatically aligned by CLUSTAL W in the software package BIOEDIT (v. 5.0.9) to give a uniform alignment length (1283 bp). Phylogenetic analysis was performed using the neighbor-joining method using the Kimura two-parameter correction model in the software MEGA (v. 3.1) (Kumar *et al.*, 2004) and bootstrap resampled 1000 times. The sequence data for the SRmet clones were assigned to operational taxonomic units (OTUs) based on a 98% sequence identity criterion. The 98% criterion was used following the argument by Wright *et al.* (2009) that the sequence similarity values between several validly recognized species of *Methanobrevibacter*; *Methanobrevibacter smithii*, *Methanobrevibacter millerae*, *Methanobrevibacter thaueri* and *Methanobrevibacter gottschalkii* were $< 2\%$, which could underestimate methanogen diversity. This is higher than the previously reported 97% value for bacteria (Stackebrandt & Goebel, 1994). Sequences are deposited in the GenBank under accession numbers EU413569–EU413665.

Results

Rumen microbial population densities

The densities (cell numbers g^{-1} wet weight) of rumen methanogenic archaea estimated using quantitative real-time PCR ranged from 1.59×10^7 to 7.11×10^8, with the mean densities decreasing by 83% from October (3.02×10^8) to April (5.16×10^7) in the Svalbard reindeer (Table 1). The mean densities of methanogens relative to bacteria ranged from 0.06% in October to 0.03% in April. Bacterial densities ranged from 1.52×10^{10} to 1.30×10^{12}, with mean values decreasing by 65% from October (5.38×10^{11}) to April (1.86×10^{11}). Numbers of ciliate protozoa ranged from 2.03×10^4 to 8.01×10^6 and the mean numbers increased 95% between October (2.03×10^5) and April (3.68×10^6). We observed considerable variation between animals, but did not find statistically significant differences between the mean microbial densities in October and April, most likely due to similar food intake levels at these times of the year (Table 1).

Table 1. Densities (cell numbers g^{-1} wet weight) of methanogenic archaea, bacteria and protozoa in the rumen of Svalbard reindeer grazing natural pasture in October (late fall) and April (late winter)

Animals	Methanogenic archaea	Bacteria	Protozoa
October			
SR1	2.14×10^7	1.83×10^{11}	2.98×10^5
SR2	3.33×10^8	1.30×10^{12}	6.13×10^5
SR3	3.51×10^8	1.23×10^{11}	2.87×10^4
SR4	7.11×10^8	4.11×10^{10}	2.03×10^4
SR5	9.34×10^7	1.04×10^{12}	5.65×10^4
Mean (SE)	3.02×10^8 (1.21×10^8)	5.38×10^{11} (2.63×10^{11})	2.03×10^5 (1.15×10^5)
April			
SR6	7.45×10^7	1.52×10^{10}	2.23×10^6
SR7	3.64×10^7	1.20×10^{11}	2.52×10^5
SR8	4.57×10^7	5.65×10^{11}	5.67×10^6
SR9	8.54×10^7	1.31×10^{11}	1.25×10^6
SR10	1.59×10^7	9.88×10^{10}	8.01×10^6
Mean (SE)	5.16×10^7 (1.27×10^7)	1.86×10^{11} (9.68×10^{10})	3.68×10^6 (1.42×10^6)
P-value	0.074	0.245	0.055

DGGE analysis of reindeer rumen microbiota

The DGGE profiles of the rumen bacterial populations did not show any obvious clustering by sampling time (Fig. 2). However, the bacterial populations of the animals grazing on October pasture (SR1–SR5) were generally more variable than those on April grazing (SR6–SR10), and there was no obvious effect of sampling time on protozoal population composition either (Fig. 3). Five of the animals (one from October pasture and four from April pasture) shared the same dominant band positions, resulting in their profiles forming a tight cluster (Fig. 3b).

Phylogenetic diversity of methanogenic archaea

The phylogeny of the dominant rumen methanogenic archaea was investigated in Svalbard reindeer grazing on October pasture through comparative analyses of 16S rRNA gene sequences ($n = 97$ clones) (Fig. 4, Table 2). A total of 22 distinct OTUs were identified using a 98% similarity criterion, which was established based on the similarity of 16S rRNA genes from characterized *Methanobrevibacter* species. Eleven of the OTUs generated from the rumen of the Svalbard reindeer exhibited a high degree of similarity to methanogens affiliated with three higher taxonomic groupings including the *Methanobacteriales* (eight OTUs, 44% of the clones), *Methanomicrobiales* (one OTU) and *Methanosarcinales* (two OTUs), while the rest of the 11 OTUs (53% of the clones) clustered with a group of uncultivated archaea previously found in other ruminants examined in recent

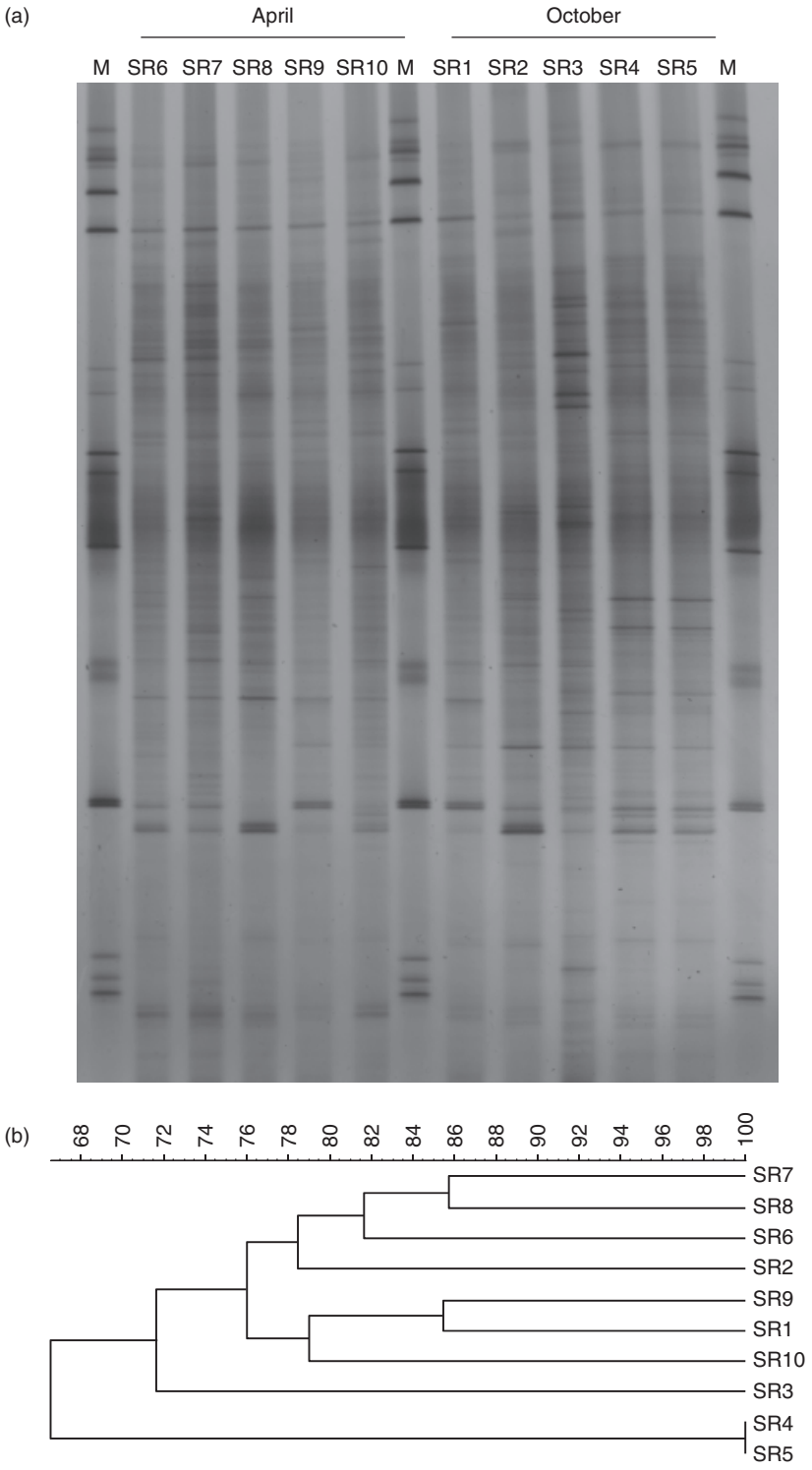

Fig. 2. DGGE profiles of bacterial 16S rRNA gene V6-8 fragments amplified using total genomic DNA extracted from rumen content samples (a), and their corresponding cluster analysis (b). Samples of whole rumen contents were collected from Svalbard reindeer grazing on October (SR1–SR5) and April (SR6–SR10) pastures. Gel markers (M) were replicated across the gel, and the similarity of their profiles (100%) was used as a threshold value for assessing differences between samples.

years, including Norwegian reindeer on summer pasture (Fig. 4). Sixteen of the OTUs (67% of the clones) showed only 80–96% identity to sequences representing the nearest validly described relative, three of the *Methanobacteriales* OTUs (28% of the clones) showed 97–98% sequence identity to *M. millerae*, one OTU (1% of the clones) showed 98% sequence identity to *M. gottschalkii*, one OTU (2% of the clones) showed 99% sequence identity to *Methanobrevibacter ruminantium* and one (2% of the clones) showed 97% sequence identity to *Methanosphaera stadtmanae* (Table 2).

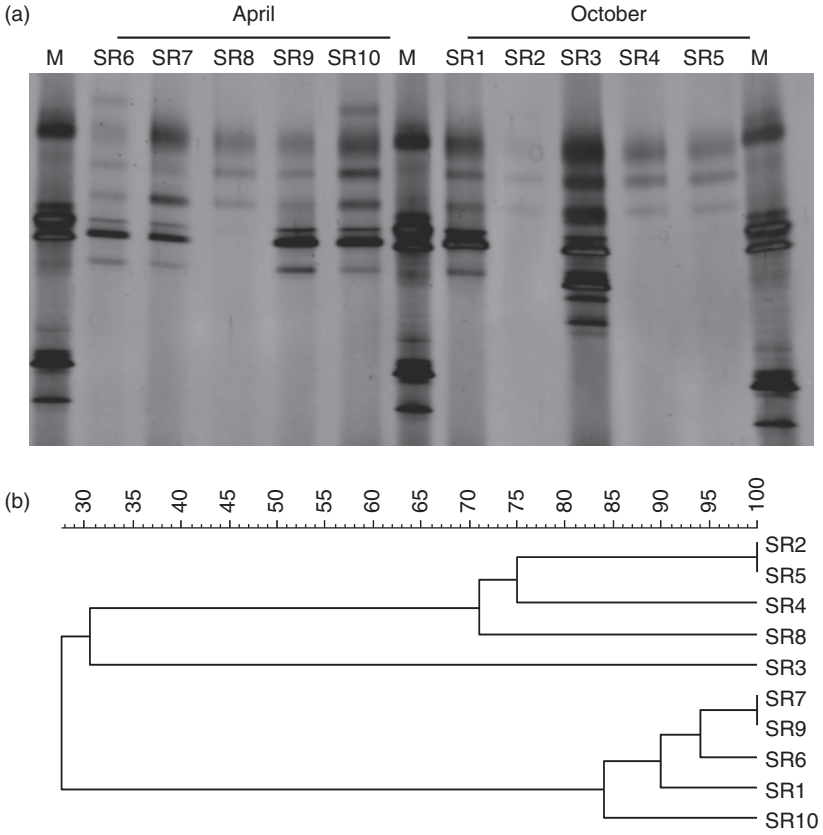

Fig. 3. DGGE profiles of rumen protozoal 18S rRNA genes amplified using total genomic DNA extracted from rumen content samples (a), and their corresponding cluster analysis (b). Samples of whole rumen contents were collected from Svalbard reindeer grazing on natural October (SR1–SR5) and April (SR6–SR10) pastures. Gel markers (M) were replicated across the gel, and the similarity of their profiles (75%) was used as a threshold value for assessing differences between samples.

Discussion

In contrast to the small variations in the diversity of rumen methanogens between reindeer and other geographically and/or genetically distant ruminants (Sundset *et al.*, 2009), studies so far indicate that the bacterial population of the reindeer is unique (Mackie *et al.*, 2003; Sundset *et al.*, 2007, 2008). In fact, the majority of the rumen bacterial 16S rRNA gene sequences retrieved from Svalbard and Norwegian reindeer were novel and did not match any known isolates or clones in GenBank (Sundset *et al.*, 2007). It is speculated that the reason for this may be associated with the harshness of their environment, in particular the extreme seasonal changes in diet, appetite and dietary intake that occur compared with what domesticated ruminants are normally exposed to.

Previous studies of Svalbard reindeer on Nordenskiöld Land at 78°N on Spitsbergen (Fig. 1), where the tundra vegetation is relatively abundant, did not reveal seasonal variations in the ruminal contents of plant cell wall organic dry matter or the mean ruminal pH (Sørmo *et al.*, 1997). However, the ruminal production rate of SCFA was low or not detectable in winter while it was 55 kJ kg^{-1} $BM^{0.75}$ day^{-1} in autumn in animals on Nordenskiöld Land (Sørmo *et al.*, 1997), reflecting the seasonal changes in their pastures.

Cultivation-based studies indicate that the numbers and composition of the rumen microorganisms in the Svalbard reindeer change with the season and chemistry of the pasture plants consumed, with large winter populations of rumen cellulolytic bacteria making their digestive system highly suitable for energy utilization of poor-quality food through slow rumen breakdown and fermentation (Mathiesen *et al.*, 2005). Rumen bacterial analysis in this study did not show any significant effect from October (late fall) to April (late winter) on the bacterial population size or composition in the Svalbard reindeer, although within the group of animals grazing the October pasture, larger differences were noted in the bacterial population composition. Appetite and activity levels are similar in October and April, but a larger variety of plant types may be available within the late fall vegetation, compared with the snow- and ice-covered winter pasture of mosses and low-quality vascular plants (Larsen *et al.*, 1985; Mathiesen *et al.*, 2005; van Oort *et al.*, 2007). It is also possible that the bacterial population structure of individual animals may be more variable than changes associated with season. Future studies with the same animals over the course of several seasons are needed to verify whether this is indeed the case. Interestingly, although bacteria are an important nitrogen source for protozoal growth, their numbers did not significantly change when ciliate numbers increased in winter.

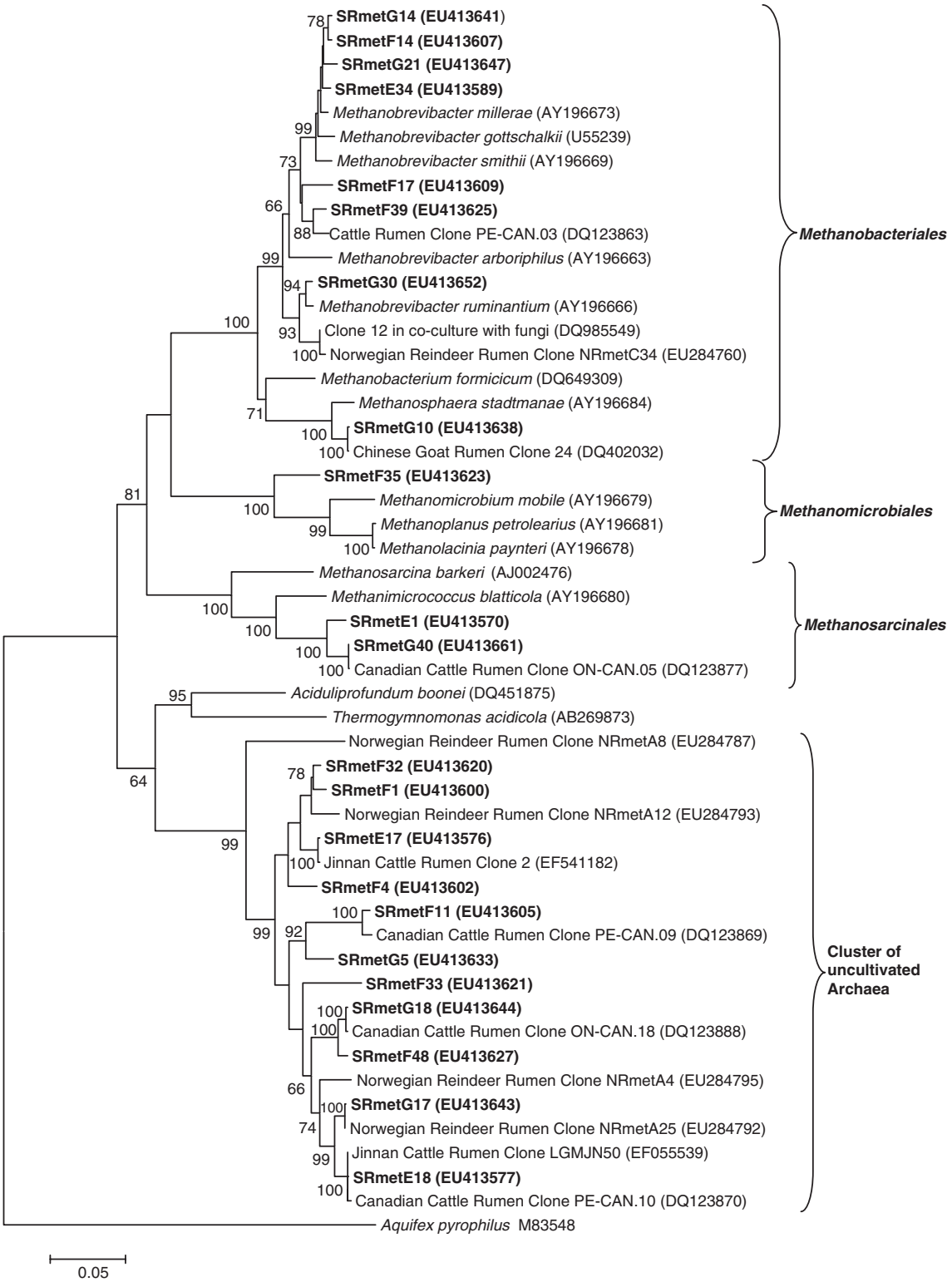

Fig. 4. Phylogenetic tree of rumen methanogenic archaeal 16S rRNA gene sequences from Svalbard reindeer (SRmet clones) representing 22 OTUs associated with the higher taxonomic groupings *Methanobacteriales*, *Methanomicrobiales*, *Methanosarcinales* and the cluster of uncultivated archaea as indicated. The scale bar represents 5% sequence divergence. Reference sequences were obtained from the GenBank database. Bootstrap values $> 60\%$ are presented. Accession numbers are given immediately following the species/strain/clone designation. Clones obtained from the rumen of the Norwegian reindeer (Sundset *et al*., 2009) are called NRmet clones. The *Aquifex pyrophilus* sequence was used as an outgroup for rooting the tree.

Table 2. Rumen methanogenic archaeal 16S rRNA gene clones from Svalbard reindeer grazing October pasture

16S rRNA gene OTUs	No. of clones*	Size (bp)	GenBank accession no.	Nearest valid taxon	Sequence identity (%)
SRmetG14	20	1234	EU413641	*Methanobrevibacter millerae*	98
SRmetF11	14	1214	EU413605	*Aciduliprofundum boonei*	81†
SEmetF17	10	1238	EU413609	*Methanobrevibacter gottschalkii*	96†
SRmetG18	9	1256	EU413644	*A. boonei*	81†
SRmetF33	7	1276	EU413621	*A. boonei*	81†
SRmetE34	6	1289	EU413589	*M. millerae*	98
SRmetE18	5	1281	EU413577	*A. boonei*	80†
SRmetG17	4	1214	EU413643	*A. boonei*	81†
SRmetE17	4	1257	EU413576	*A. boonei*	82†
SRmetF4	4	1285	EU413602	*Thermogymnomonas acidicola*	82†
SRmetG10	2	1219	EU413638	*Methanosphaera stadtmanae*	97†
SRmetG30	2	1213	EU413652	*Methanobrevibacter ruminantium*	99
SRmetF1	1	1286	EU413600	*A. boonei*	83†
SRmetG5	1	1228	EU413633	*A. boonei*	81†
SRmetF48	1	1268	EU413627	*A. boonei*	81†
SRmetF35	1	1287	EU413623	*Methanomicrobium mobile*	93†
SRmetF32	1	1230	EU413620	*Thermogymnomonas acidicola*	82†
SRmetG40	1	1268	EU413661	*Methanimicrococcus blatticola*	92†
SRmetE1	1	1258	EU413570	*M. blatticola*	91
SRmetG21	1	1219	EU413647	*M. millerae*	97†
SRmetF39	1	1246	EU413625	*M. gottschalkii*	95†
SRmetF14	1	1296	EU413607	*M. gottschalkii*	98

*In total 97 clones were examined.

†Potential new species based on the 98% identity criterion.

Svalbard reindeer survive the winter by reducing their energy expenditure to a minimum, and mobilizing fat reserves (Mathiesen *et al.*, 2005). Previous studies by Tyler (1987) showed that the carcass mass of Svalbard reindeer reduced from 72 kg in summer to 46 kg in winter when they have to mobilize a large proportion of their protein and energy reserves. Additionally, a large rumen with high concentrations of cellulolytic bacteria (Orpin *et al.*, 1985), and probably longer retention times, may represent an adaptation that allows the Svalbard reindeer to optimize their ruminal utilization of low-quality fibrous food devoid of lichens (Mathiesen *et al.*, 2005). These factors may provide conditions that favor populations of ciliate protozoa.

Since the publication of data on ruminal methanogen population sizes in Norwegian reindeer (Sundset *et al.*, 2009), the methanogen real-time primers Met630F and Met803R were also recently found to amplify bacteria under some conditions, potentially overestimating the numbers of methanogens (A.-D.G. Wright, pers. commun.). This may explain the considerably higher numbers of rumen methanogens reported in Norwegian reindeer (Sundset *et al.*, 2009) compared with those reported for Svalbard reindeer in this study, which were generated using primers targeting the mcrA gene (only found in methanogenic archaea). Quantification of the ruminal methanogens in Norwegian reindeer was repeated using the mcrA based real-time PCR method (Table 3), and methanogen numbers were indeed found to be significantly lower than those published previously (Sundset *et al.*, 2009). This finding therefore indicates that ruminal methanogen population sizes are comparable in both Svalbard and Norwegian reindeer.

Table 3. Densities (cell numbers g^{-1} wet weight) of methanogenic archaea in Norwegian reindeer grazing natural summer pasture

Animals*	Methanogenic archaea
NR1	4.65×10^8
NR2	1.54×10^8
NR3	5.92×10^8
NR4	1.71×10^8
NR5	6.22×10^8
Mean (SE)	4.01×10^8 (1.01×10^8)

*Animals and sampling described by Sundset *et al.* (2009).

Despite similar methanogen population sizes, the ruminal protozoal population size in Svalbard reindeer (2.03×10^5 cells g^{-1} wet weight) was much lower than that in Norwegian reindeer (4.02×10^7 cells g^{-1} wet weight) (Sundset *et al.*, 2009). A decrease in the numbers of ciliate protozoa would normally be expected to be associated with a decline in the numbers of methanogens, as many of the ruminal methanogens live in close association with protozoa (Vogels *et al.*, 1980; Regensbogenova *et al.*, 2004). It is

therefore likely that a significant proportion of the methanogens present in the rumen of Svalbard reindeer are not closely associated with ciliate protozoa. In domestic ruminants, removal of ruminal protozoa can result in a decrease in methane emissions of up to 40% (Finlay *et al.*, 1994; Newbold *et al.*, 1995; Dohme *et al.*, 1999).

Little was previously known about the population composition of rumen methanogens in Svalbard reindeer, and how they compared with Norwegian reindeer (Sundset *et al.*, 2009). The findings of this study indicate that the dominant methanogens in both types of reindeer are similar, although the proportion of uncultivated ruminal archaeal 16S rRNA gene sequences [belonging to the previously described Rumen Cluster C (Janssen & Kirs, 2008)] was much higher in the Svalbard reindeer (53%) compared with the Norwegian reindeer (17%). As these methanogens are not culturable using standard approaches, this observation may also partly explain why previous cultivation-based numbers from Svalbard reindeer [10^4–10^7 cells mL^{-1} rumen fluid (Orpin *et al.*, 1985)] are lower than the numbers obtained by molecular approaches (10^7–10^8 cells g^{-1} wet weight). This analysis confirms that the population densities of rumen methanogenic archaea in Svalbard and Norwegian reindeer (presented in this paper) are lower than those typically found in domestic ruminants such as sheep (10^8–10^9; Skillman *et al.*, 2004) and cattle (up to 1.34×10^9; Denman *et al.*, 2007).

Host type appears to have a significant effect on rumen methanogen population size, with reindeer having less methanogens than domesticated sheep and cattle. The similarity of the rumen archaeal 16S rRNA gene sequences obtained from the Svalbard and Norwegian reindeer to those reported in geographically distant domestic ruminants (Tajima *et al.*, 2001; Wright *et al.*, 2007, 2008; Ohene-Adjei *et al.*, 2008) supports previous findings that there seems to be no host type or geographical effect on the species distribution of ruminal methanogens.

In conclusion, the geographically isolated Svalbard reindeer are unique ruminants that can provide insights into the adaptation and resilience of ruminal microbiota in wild ruminants. No consistent differences were detected in the microbial population composition of protozoa and bacteria between reindeer on late autumn pasture in October and late winter pasture in April when food intakes are at similar levels, while animal variations were considerable. Similarly, microbial numbers did not change significantly between October and April, although there was a tendency for the methanogenic archaea to be higher in October than in April ($P = 0.074$), and the ciliate numbers to be lower ($P = 0.055$). Although reindeer have much smaller populations of rumen methanogens than domesticated sheep and cattle, methane emissions from both domestic and wild reindeer populations across the world (estimated to about 5 million animals) remain to be measured.

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