The Journal of Microbiology (2011) Vol. 49, No. 3, pp. 492-498
DOI 10.1007/s12275-011-0319-7

NOTE

Diversity of Bovine Rumen Methanogens *In Vitro* in the Presence of Condensed Tannins, as Determined by Sequence Analysis of 16S rRNA Gene Library

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(Received August 16, 2010 / Accepted January 5, 2011)

Molecular diversity of rumen archaeal populations from bovine rumen fluid incubated with or without condensed tannins was investigated using 16S rRNA gene libraries. The predominant order of rumen archaea in the 16S rRNA gene libraries of the control and condensed tannins treatment was found to belong to a novel group of rumen archaea that is distantly related to the order *Thermoplasmatales*, with 59.5% (15 phylotypes) and 81.43% (21 phylotypes) of the total clones from the control and treatment clone libraries, respectively. The 16S rRNA gene library of the control was found to have higher proportions of methanogens from the orders *Methanomicrobiales* (32%) and *Methanobacteriales* (8.5%) as compared to those found in the condensed tannins treatment clone library in both orders (16.88% and 1.68% respectively). The phylotype distributed in the order *Methanosarcinales* was only found in the control clone library. The study indicated that condensed tannins could alter the diversity of bovine rumen methanogens.

***Keywords*:** condensed tannins, methanogens, bovine rumen fluid, rumen archaeal diversity, gene library

Global greenhouse gas emissions have grown with an increase of 70% from the 1970s to 2004 (IPCC, 2007). Methane (CH_4) is the second most important greenhouse gas that contributes to global warming and climate change. Methane production from livestock, predominantly ruminants, accounts to about one-third of global anthropogenic methane production (US-EPA, 2006). It has been projected by FAO that CH_4 production is expected to increase by 60% by the year 2030, if the emission grows in direct proportion to the increase in livestock numbers (FAO, 2003). Methane production during ruminal fermentation also contributes to a loss of feed energy of up to 12%. Therefore, methane mitigation has become a central issue in ruminant production.

Methane is a normal product of rumen fermentation and it is an end-product for the disposal of metabolic hydrogen in an anaerobic system in the rumen. Methanogenic archaea play a major role in the reduction of carbon dioxide and hydrogen to methane, and because of this, variations in the diversity of methanogen communities in the rumen have received considerable attention (Whitford *et al*., 2001; Wright *et al*., 2006, 2007).

Methane mitigations through the incorporation of anti-methanogenic compounds such as bromochloromethane (Denman *et al*., 2007), monensin (Hook *et al*., 2009), and saponin (Hess *et al*., 2003) in the diets of ruminants have shown variations in the diversity of methanogen populations. Condensed tannins from forages have been reported to reduce methane production in ruminants (Woodward *et al*., 2001; Waghorn *et al*., 2002; Tavendale *et al*., 2005) but there is no information on the rumen methanogen population when condensed tannins are supplemented. Thus, this study was conducted to investigate the diversity of rumen methanogens in the presence or absence (control) of condensed tannins (from *Leucaena leucocephala* hybrid-Rendang) *in vitro* using sequence analysis of 16S rRNA gene library. To the best of our knowledge, this is the first investigation pertaining to the diversity of rumen methanogens in the presence of condensed tannins.

Pure condensed tannins were extracted and purified from young shoots and leaves of a *L. leucocephala* hybrid-Rendang (LLR) using the method of Terrill *et al*. (1990, 1992). Three rumen-fistulated steers of Kedah-Kelantan bred (*Bos indicus*) with an average weight of 209 kg were used as the donors of rumen digesta. All animal management and sampling procedures were approved by the Universiti Putra Malaysia Animal Care and Use Committee. The animals were fed with a fixed amount of guinea grass (*Panicum maximum*) and concentrates (60:40) twice daily based on the grass to concentrate ratio at 2.5% of BW/day. Rumen digesta was collected from each animal via the ruminal fistula before morning feeding. The rumen digesta samples were pooled in equal proportions, then strained through four layers of cheesecloth and the resulting rumen fluid was incubated with condensed tannins using the *in vitro* gas production test of Menke and Steingass (1988) with modifications by Makkar (1995). The *in vitro* gas production test is commonly used for initial evaluation of effects

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of compounds on rumen microbial activities as it is easy to manage, rapid, and more cost effective than *in vivo* studies involving live ruminants. In the test, the clarified rumen fluid was first mixed with three parts of pre-warmed buffer solution at 39°C, then 40 ml of the mixture was dispensed into 100 ml calibrated glass syringes (Häberle Labortechnik, Germany) and incubated with 0 mg (control) or 20 mg of condensed tannin extract, and 500 mg of guinea grass for 24 h at 39°C. It had been found that 20 mg of condensed tannin extract was the optimum concentration for reduction of methane; the methane and total gas productions were decreased by 34.0% and 34.3%, respectively, with no significant ($P<0.05$) reduction in the dry matter digestibility of feed when compared to the control (Tan, H.Y., unpublished data). All the procedures were carried out under anaerobic conditions. Three replications were made for the *in vitro* gas production test.

After the incubation period, 300 µl of the rumen fluid mixture was used for extraction of total DNA using the QIAamp® DNA Stool kit (QIAGEN, Germany). The procedure was carried out in triplicate.

The PCR amplification conditions were according to Wright *et al*. (2004) with some modifications. Methanogen-specific forward and reverse primers, Met 86F and Met 1340R (Wright and Pimm, 2003), were used for PCR amplification with an Apollo ATC 401 Thermal Cycler (UVItec Limited, UK). PCR reactions were performed in a mixture containing 1 µl of genomic DNA as the template, 200 nM of each primer, 1× VioTaq® reaction buffer, 10 µM of each dNTP (i-DNA Biotechnology Pte Ltd, Singapore), 0.5 U of VioTaq® Taq DNA polymerase (Viogene, Taiwan) and de-ionized water in a 20 µl reaction. The amplification conditions were: initial denaturation of 5 min at 94°C followed by 40 cycles of denaturation for 30 sec at 94°C, 1 min of primer annealing at 58°C, 90 sec of primer extension at 72°C and a primer extension for 10 min at the last cycle.

The expected amplicon of about 1.3 kb was excised after gel electrophoresis and purified using a MEGAquick-spin™ PCR & Agarose Gel DNA Extraction kit (iNtRON Biotechnology, Korea). The purified PCR product was ligated into pCR 2.1® TOPO vector using a PCR 2.1® TOPO TA Cloning kit (Invitrogen Ltd, USA) and cloned into *E. coli* TOP 10. Positive clones were randomly selected and subjected to plasmid extraction (DNA-spin™ Plasmid DNA Extraction kit, iNtRON Biotechnology). The recombinant plasmids were digested with restriction endonuclease *Hae*III (Wright and Pimm, 2003). Restriction fragment length polymorphisms were grouped according to their riboprint patterns. Representative clones from each phylotype were sequenced for identity confirmation. Sequencing was performed with an automated sequencer ABI 3730 XL using Big Dye Chemistry.

Potential anomalous sequences were then examined by the Mallard program (Ashelford *et al*., 2005). Sequences were assembled in Bioedit sequence alignment editor (Hall, 1999). The methanogen sequences were compared with the sequences available in the GenBank database (Benson *et al*., 2008) by using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) to determine sequence homology. A sequence similarity of 95% or less (Amann *et al*., 1995) was considered as a new archaeon species.

Twenty-four methanogen sequences from the GenBank database were used as the reference sequences. The nucleotide sequences were under the following accession numbers: *Metha-*

Table 1. Similarity values of methanogen 16S rRNA gene sequences from rumen fluid incubation without condensed tannins (control)

16S rRNA phylotype	No. of clones	Size	GenBank accession no.	Nearest valid taxon	% sequence similarity
MC 01	17	1256	HQ616003	*Thermoplasma volcanium*	80
MC 02	13	1256	HQ616004	*Thermoplasma* sp.	80
MC 03	44	1257	HQ616005	*Thermoplasma volcanium*	79
MC 04	21	1255	HQ616006	*Methanomicrobium mobile*	99
MC 05	6	1256	HQ616007	*Methanococcoides* sp.	78
MC 06	19	1256	HQ616008	*Thermoplasma acidophilum*	79
MC 07	1	1257	HQ616009	*Thermoplasma volcanium*	79
MC 08	40	1257	HQ616010	*Methanomicrobium mobile*	99
MC 09	1	1258	HQ616011	*Thermoplasma acidophilum*	79
MC 10	4	1256	HQ616012	*Thermoplasma volcanium*	80
MC 11	1	1259	HQ616013	*Thermoplasma volcanium*	78
MC 12	10	1261	HQ616014	*Methanobrevibacter millerae*	98
MC 13	1	1256	HQ616015	*Thermoplasma volcanium*	78
MC 14	1	1256	HQ616016	*Methanomicrobium mobile*	99
MC 15	1	1257	HQ616017	*Methanobrevibacter* sp.	99
MC 16	4	1262	HQ616018	*Methanobrevibacter millerae*	98
MC 17	1	1256	HQ616019	*Thermoplasma volcanium*	79
MC 18	1	1256	HQ616020	*Thermoplasma acidophilum*	79
MC 19	2	1260	HQ616021	*Methanobrevibacter* sp.	99
MC 20	1	1257	HQ616022	*Thermoplasma volcanium*	79
MC 21	2	1255	HQ616023	*Methanomicrobium mobile*	99
MC 22	1	1258	HQ616024	*Methanimicrococcus blaticticola*	91
MC 23	8	1256	HQ616025	*Thermoplasma acidophilum*	79

A total of 200 clones were examined

nolobus taylorii (U20154), *Methanosarcina barkeri* (AJ012094), *Methanimicrococcus blatticola* (AJ238002), *Methanoplanus limicola* (M59143), *Methanoplanus petrolearius* (U76631), *Methanomicrobium mobile* (M59142), *Methanobacterium formicicum* (AF169245), *Methanosphaera stadtmanae* (AY196684), *Methanobrevibacter curvatus* (U62533), *Methanobrevibacter filiformis* (U82322), *Methanobrevibacter cuticularis* (U41095), *Methanobrevibacter acididurans* (AF242652), *Methanobrevibacter olleyae* (AY615201), *Methanobrevibacter ruminantium* (AY196666), *Methanobrevibacter wolinii* (U55240), *Methanobrevibacter woesei* (U55237), *Methanobrevibacter smithii* (CP000678), *Methanobrevibacter thaueri* (U55236), *Methanobrevibacter gottschalkii* (U55238), *Methanobrevibacter millerae* (AY196673), *Methanococcus vannielii* (AY196675), *Picrophilus oshimae* (X84901), *Thermoplasma volcanium* (AJ299215), and *Thermoplasma acidophilum* (M38637). *Sulfolobus acidocaldarius* (D14053) and *Thermoproteus tenax* (AY538162) were used as the outgroups.

All sequences were globally aligned using the multiple sequence alignment program CLUSTAL W (Thompson *et al*., 1994). Evolutionary distances between pairs of nucleotide sequences were calculated using the Kimura two-parameter model (Kimura, 1980). A phylogenetic tree was constructed by Molecular Evolutionary Genetic Analysis (MEGA) version 4.0 (Tamura *et al*., 2007) based on the neighbor-joining algorithm (Saitou and Nei, 1987) and was bootstrap (Felsenstein, 1985) resampled 1,000 times.

The Shannon index (*H*) (Shannon and Weaver, 1949) was used to characterize species diversity between the two clone libraries. Sequences from the 16S rRNA gene of the control clone library were designated with the prefix MC while sequences from the 16S rRNA gene of the condensed tannins treatment clone library were designated with the prefix MLR. These prefixes were followed by the number of the unique phylotype.

A total of 53 nucleotide sequences from the two 16S rRNA gene libraries obtained in this study have been deposited in the GenBank database under the accession numbers HQ616003 to HQ616025 (control) and HM038363 to HM038392 (condensed tannins treatment).

In the first 16S rRNA gene library, which was from the control treatment, a total of 204 clones were examined, of which four clones were identified as chimeras and excluded from the analysis. The remaining 200 clones revealed 23 different phylotypes (Table 1). In the second 16S rRNA gene library, which was from the condensed tannins treatment, a total of 244 clones were randomly selected. Riboprint pattern analysis from the total clones revealed 37 different phylotypes, but seven phylotypes (one clone each) were identified

Table 2. Similarity values of methanogen 16S rRNA gene sequences from rumen fluid incubation with condensed tannins

16S rRNA phylotype	No. of clones	Size	GenBank accession no.	Nearest valid taxon	% sequence similarity
MLR 01	81	1256	HM038363	*Thermoplasma acidophilum*	79
MLR 02	7	1256	HM038364	*Thermoplasma volcanium*	79
MLR 03	2	1254	HM038365	*Methanomicrobium mobile*	99
MLR 04	14	1255	HM038366	*Methanomicrobium mobile*	99
MLR 05	5	1256	HM038367	*Thermoplasma* sp.	79
MLR 06	1	1256	HM038368	*Thermoplasma volcanium*	79
MLR 07	2	1257	HM038369	*Thermoplasma volcanium*	79
MLR 08	1	1255	HM038370	*Thermoplasma acidophilum*	79
MLR 09	12	1255	HM038371	*Methanomicrobium mobile*	99
MLR 10	2	1260	HM038372	*Methanobrevibacter millerae*	98
MLR 11	2	1256	HM038373	*Thermoplasma acidophilum*	78
MLR 12	1	1256	HM038374	*Methanolobus* sp.	79
MLR 13	1	1256	HM038375	*Thermoplasma acidophilum*	79
MLR 14	1	1256	HM038376	*Thermoplasma acidophilum*	78
MLR 15	1	1257	HM038377	*Thermoplasma volcanium*	79
MLR 16	4	1255	HM038378	*Thermoplasma volcanium*	79
MLR 17	1	1255	HM038379	*Methanomicrobium mobile*	99
MLR 18	1	1256	HM038380	*Thermoplasma volcanium*	79
MLR 19	1	1257	HM038381	*Methanobrevibacter millerae*	98
MLR 20	1	1256	HM038382	*Thermoplasma volcanium*	79
MLR 21	1	1256	HM038383	*Thermoplasma acidophilum*	79
MLR 22	2	1254	HM038384	*Methanomicrobium mobile*	99
MLR 23	1	1260	HM038385	*Methanobrevibacter ruminantium*	98
MLR 24	1	1256	HM038386	*Thermoplasma volcanium*	79
MLR 25	68	1256	HM038387	*Thermoplasma acidophilum*	79
MLR 26	1	1256	HM038388	*Methanolobus* sp.	79
MLR 27	9	1255	HM038389	*Methanomicrobium mobile*	99
MLR 28	9	1256	HM038390	*Methanolobus* sp.	78
MLR 29	3	1259	HM038391	*Picrophilus oshimae*	79
MLR 30	1	1259	HM038392	*Methanococcoides* sp.	77

A total of 237 clones were examined

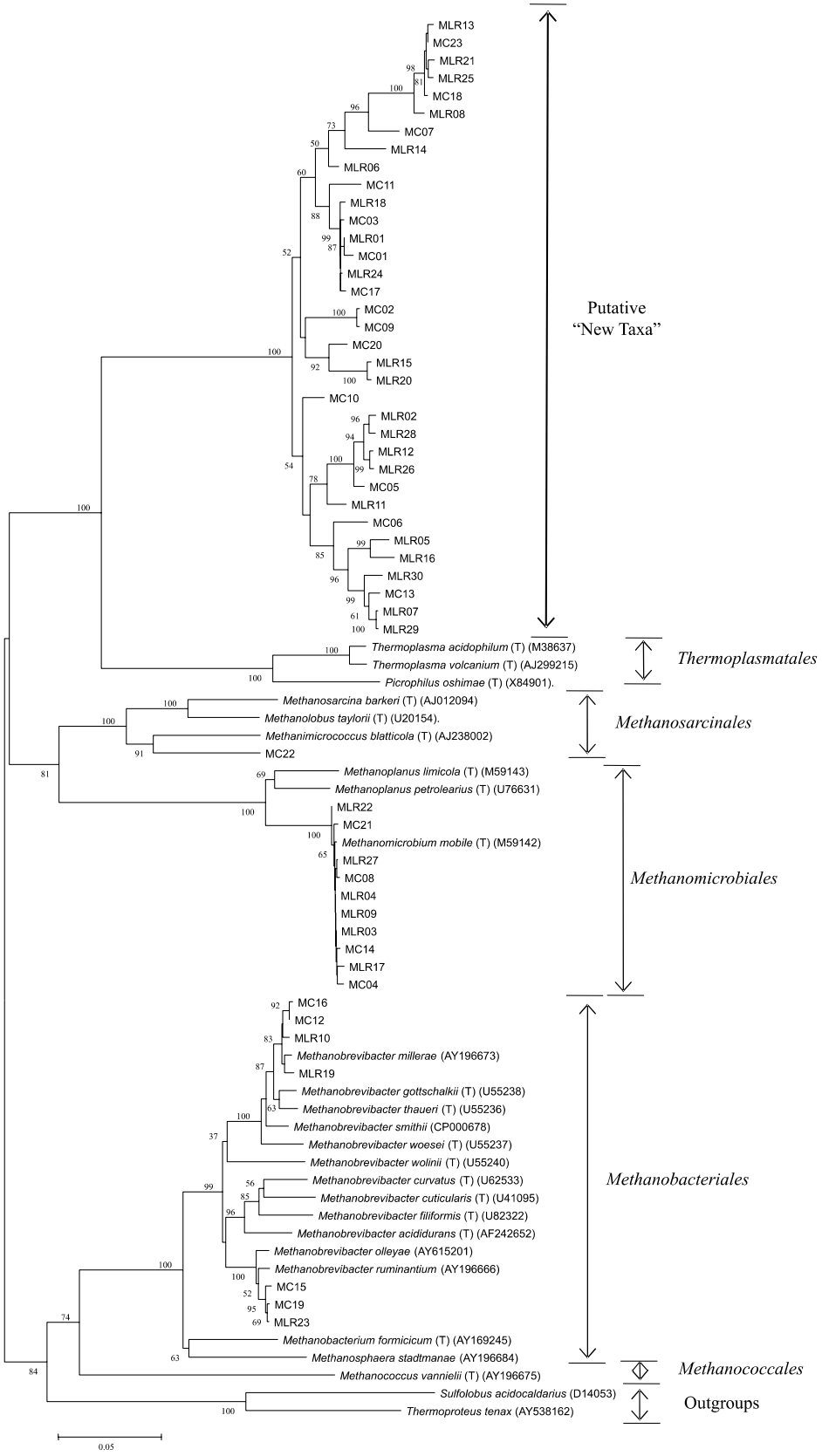

Fig. 1. Phylogenetic relationship of archaeal clones derived from 16S rRNA gene evolutionary distances constructed using the neighbor joining method. The scale bar represents 0.05-nucleotide substitutes per position.

as chimeras and excluded from the data. The remaining 237 clones were represented by 30 phylotypes (Table 2). Based on the Shannon diversity index (*H*), it appeared that the clone library of the control treatment exhibited more methanogen diversity (H=2.399) than the clone library of the condensed tannins treatment (H=2.149).

The results from the BLAST analysis for the control clone library showed that 15 phylotypes (119 clones) or 59.5% of the total clones were not closely related to any recognized cultivated archaeal taxa and were identified as putative new taxa based on the similarity criterion of <95% for new species (Amann *et al*., 1995). Tables 1 and 2 show the nearest valid taxon of the cultivated methanogens that have been published.

Among the 15 phylotypes of the putative new taxa in the 16S rRNA gene sequences of the control clone library, 13 phylotypes (MC01, MC02, MC03, MC06, MC07, MC09, MC10, MC11, MC13, MC17, MC18, MC20, and MC23) showed 78 to 80% similarity to *Thermoplasma acidophilum*, *T. volcanium*, and *Thermoplasma* sp. from the order *Thermoplasmatales* (Table 1). The other two phylotypes (MC05 and MC22) were distantly related to the order *Methanosarcinales*, with MC05 exhibiting 78% similarity to *Methanococcoides* sp. and MC22 showing 91% similarity to *Methanimicrococcus blatticola*.

The remaining 40.5% of the total clones from the control clone library exhibited similarity to the archaeal taxa from the orders *Methanomicrobiales* and *Methanobacteriales*. Four phylotypes (MC04, MC08, MC14, and MC21) (64 clones) which represented 32% of the total clones were 99% similar to *Methanomicrobium mobile*. Another four phylotypes (17 clones) were at least 98% similar to archaea in the order *Methanobacteriales*, with two phylotypes (MC15 and MC19) showing 99% similarity to *Methanobrevibacter* sp. and another two phylotypes (MC12 and MC16) exhibiting 98% similarity to *Methanobrevibacter millerae*.

In the condensed tannins treatment clone library, 21 phylotypes (193 clones), which accounted for 81.43% of the total clones, were related to the putative new archaeal taxa. Of the 21 phylotypes, 17 phylotypes were putative new taxa distantly related to the order *Thermoplasmatales* and four phylotypes to the order *Methanosarcinales* (Table 2). Of the 17 *Thermoplasmatales*-associated phylotypes, one phylotype (MLR29) was 79% similar to *Picrophilus oshimae* and the other 16 phylotypes (MLR01, MLR02, MLR05, MLR06, MLR07, MLR08, MLR11, MLR13, MLR14, MLR15, MLR16, MLR18, MLR20, MLR21, MLR24, and MLR25) were 78 to 79% similar to *T. acidophilum*, *T. volcanium*, and *Thermoplasma* sp. Of the four phylotypes that were distantly related to the order *Methanosarcinales*, three (MLR12, MLR26, and MLR28) were 78-79% similar to *Methanolobus* sp. and one (MLR30) was 77% similar to *Methanococcoides* sp.

A minor proportion of the sequences (44 clones, 18.56% of total clones) had high degree of sequence similarity (98-99%) with three validly recognized methanogen species. These 44 clones belonged to nine phylotypes. Two of the phylotypes, MLR10 and MLR19 (three clones), were 98% similar to *Methanobrevibacter millerae* and one phylotype, MLR23 (one clone), was 98% similar to *Methanobrevibacter ruminantium*. Another six phylotypes, MLR03, MLR04, MLR09, MLR17, MLR22, and MLR27 (40 clones), were 99% similar to *Methanomicrobium mobile*.

Phylogenetic analysis of sequences to define their taxonomic positions revealed that the majority of the sequences were clustered into the first clade of the phylogenetic tree (Fig. 1). The 14 phylotypes (118 clones) from the control clone library and 21 phylotypes (193 clones) from the condensed tannins treatment clone library, which were putative new taxa, were grouped together and were monophyletic with the order *Thermoplasmatales* with a high bootstrap value of 100%. This group formed a distinct clade branching with a sister group containing the thermoacidophilic scavengers, *T. acidophilum*, *T. volcanium*, and *Picrophilus oshimae*, in the order *Thermoplasmatales*.

There were ten phylotypes which phylogenetically grouped within the order *Methanomicrobiales*. Of these, three phylotypes of the control clone library (MC04, MC08, and MC14; 62 clones) and five phylotypes of the condensed tannins treatment clone library (MLR03, MLR04, MLR09, MLR17, and MLR27; 38 clones), were clustered together with *M. mobile* with a remarkable bootstrap value of 100%. One phylotype from each library, MC21 (2 clones) and MLR22 (2 clones), formed a sister group to *M. mobile* and branched basal to the clade consisting of the other eight phylotypes (MC04, MC08, MC14, MLR03, MLR04, MLR09, MLR17, MLR27).

Within the order *Methanobacteriales*, one phylotype, MLR19 (1 clone) from the condensed tannins treatment clone library, and *Methanobrevibacter millerae* formed a monophyletic unit. Phylotypes MC12 (10 clones) and MC16 (4 clones) from the control clone library, and MLR10 (2 clones) from the condensed tannins treatment clone library were clustered with *M. millerae* and MLR19 with a bootstrap value of 83%. Another three phylotypes, MC15 (1 clone) and MC19 (2 clones) from the control clone library, and MLR23 (1 clone) from the condensed tannins treatment clone library, formed a monophyletic clade with *Methanobrevibacter ruminantium* and these methanogen species formed a sister group to *Methanobrevibacter olleyae* with a high bootstrap value of 100%.

One phylotype, MC22 (1 clone) from the control clone library formed a monophyletic unit with *Methanimicrococcus blatticola* with a high bootstrap value of 91%.

The present results, which showed that the majority of the total clones from bovine rumen fluid incubated with or without condensed tannins were novel 16S rRNA gene sequences of archaea distantly related to the order *Thermoplasmatales*, were similar to those obtained by other researchers for cattle and sheep. Tajima *et al*. (2001) studied bovine rumen archaeal diversity using two different sets of archaeal primers and found that 79% of the clones from one gene library and 20% of the clones from another library were distantly associated with *Thermoplasma*. Wright *et al*. (2006) also found that over 80% of the total clones (63 of 78 clones) from the rumen of sheep in Australia were a putative new order related to the *Thermoplasmatales*. Later, Wright *et al*. (2007) reported that 50% of the clones from 16S rRNA gene library of potato-fed cattle clustered with the clone sequences of uncultured archaea with 74.1 to 75.8% similarity to *Thermoplasma acidophilum* and *T. volcanium*. Archaeal sequences clustering within the *Thermoplasmatales* have also been found from the hindgut of termite (Shinzato *et al*., 1999). Cultivated known members of the *Thermoplasmatales* are found to be non-methanogenic

and lack the genes coding for proteins involved in the methanogenesis pathway (Bapteste *et al*., 2005). *Thermoplasma* has adapted to scavenging nutrients from the decomposition of microbial biomass (Ruepp *et al*., 2000). Without cultivated isolates of the novel *Thermoplasmatales*-associated archaea discovered in the present study, their role in the rumen remains unclear, since the cultivated known members are atypical of a rumen environment.

In the current study, 16.9% (40 clones) of the total clones from the condensed tannins treatment clone library resembled *Methanomicrobium mobile* (similarity value of 99%). In contrast, in the control clone library, about one third or 32% of the total clones were similar to *M. mobile*. This result is similar to that of Shin *et al*. (2004) in which 95.6% of the total clones from bovine rumen fluid belonged to the order *Methanomicrobiales* with 33.3% resembling *M. mobile*. Tajima *et al*. (2001) also found 56% of the clones to be *M. mobile*-associated in a clone library from bovine rumen fluid. *M. mobile*-like clones have been found in rumen of cattle and sheep (Lin *et al*., 1997; Yanagita *et al*., 2000; Irbis and Ushida, 2004; Regensbogenova *et al*., 2004). In buffaloes, 94.4% of the total clones were associated with *M. mobile* (Chaudhary and Sirohi, 2009). However, some investigations reported an absence of sequences closely related to *Methanomicrobium* in bovine rumen (Whitford *et al*., 2001; Skillman *et al.*, 2004; Wright *et al*., 2007). In ovine rumen only 7.7% (six clones of 78 clones examined) resembled *M. mobile* (Wright *et al*., 2006).

Several studies have shown that *Methanobrevibacter* is the predominant genus in the rumen of cattle and sheep (Lin *et al*., 1997; Sharp *et al*., 1998; Whitford *et al*., 2001; Skillman *et al*., 2004; Wright *et al*., 2004, 2008; Pei *et al*., 2009). However, the current findings indicated that only 8.5% (17 clones) and 1.7% (4 clones) of the clones from the control and condensed tannins treatment clone library, respectively, were in the order *Methanobacteriales*, with four phylotypes (MC12, MC16, MLR10, and MLR 19) associated with *M. millerae*, two (MC15 and MC19) with *Methanobrevibacter* sp. and one (MLR23) with *Methanobrevibacter ruminantium*. Tajima *et al*. (2001) have also reported that *Methanobrevibacter* spp. were not predominant (16% of the total clones) in bovine rumen fluid.

The current investigation indicated that there were some differences in the density and diversity of bovine rumen methanoges in the clone libraries of the control and condensed tannins treatment. The inclusion of 20 mg of condensed tannins in bovine rumen fluid reduced the population of methanogens in the orders *Methanomicrobiales* and *Methanobacteriales* by 15.1% and 6.8%, respectively, as compared to the control. In contrast, the population of putative new taxa distantly related to the order *Thermoplasmatales* increased by 21.9% in the presence of condensed tannins (from 59.5% in the control clone library to 81.4% in the condensed tannins treatment clone library). Methanogens from the order *Methanosarcinales* were not detected in the presence of condensed tannins. The higher Shannon Diversity Index (*H*) of the control clone library indicated that it had a greater diversity of methanogens than the condensed tannins treatment clone library.

The current study revealed for the first time the molecular diversity of bovine rumen archaeal community in the presence of condensed tannins. As condensed tannins from forages have been found to reduce methane production in ruminants, more studies are needed to better understand the effect of these plant metabolites on the rumen methanogen population and explore ways to manipulate them for methane mitigation in ruminants.

Acknowledgements

This study was supported by the Research University Grant Scheme, Universiti Putra Malaysia: Project No. 04/01/07/0075RU.

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