



Effects of condensed tannin fractions of different molecular weights on population and diversity of bovine rumen methanogenic archaea *in vitro*, as determined by high-throughput sequencing

Mookiah Saminathan^a, Chin Chin Sieo^{a,b}, Han Ming Gan^{c,d},
Norhani Abdullah^{b,e,*}, Clemente Michael Vui Ling Wong^f, Yin Wan Ho^a

^a Institute of Bioscience, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia

^b Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia

^c School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Selangor, Malaysia

^d Genomics Facility, Tropical Medicine Biology Platform, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500 Selangor, Malaysia

^e Institute of Tropical Agriculture, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia

^f Biotechnology Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah 88999, Malaysia

ARTICLE INFO

Article history:

Received 8 May 2015

Received in revised form 23 February 2016

Accepted 5 April 2016

Keywords:

Condensed tannin fractions

Molecular weight

Leucaena leucocephala

Methanogen population

Archaeal diversity

Illumina Miseq

ABSTRACT

It has been suggested that condensed tannin (CT) fractions of different molecular weights (MWs) affect the level of methane (CH₄) production in ruminant by altering the ruminal methanogenic diversity. In this study, the effects of unfractionated CTs (F0) and five CT fractions (F1–F5) of different MWs from *Leucaena leucocephala* hybrid-Rendang (LLR) on the population and diversity of rumen methanogenic archaea *in vitro* were investigated using real-time PCR and high throughput sequencing. Real-time PCR analysis showed that higher-MW CT fractions (fractions F1 and F2) significantly ($P < 0.05$) decreased more total methanogens and methanogens from the order *Methanobacteriales* than the control (without CTs), unfractionated CTs F0 and lower-MW CT fractions (fractions F3–F5). Population of methanogens from the order *Methanomicrobiales* was not significantly ($P > 0.05$) different among all CT fractions and the control. Amplification of archaeal 16S RNA genes (targeting the V3 region) of seven samples (obtained from *in vitro* gas production test) using Illumina Miseq sequencer, recovered 642,244 sequences (mean of 91,763 sequences per sample) which consisted of a number of OTUs ranging from 91 to 155 and were assigned to 12 genera. Bioinformatic analysis illustrated that the natural rumen archaeal community of a cattle (without supplementation of CTs) was predominated by an unclassified genus, VadinCA11 gut group which is *Thermoplasmata*-associated (56.3% of the total sequence reads), followed by *Methanobrevibacter* (25.1%) and *Methanomicrobium* (15.3%), while the remaining genera were below 1% of relative abundance. Relative abundance of the unclassified *Thermoplasmata*-associated group (VadinCA11 gut group) increased significantly ($P < 0.05$) with increasing MWs of the CT fractions, whereas the predominant methanogen genus *Methanobrevibacter* was significantly ($P < 0.05$) decreased. Condensed tannin fraction F4, but not the other CT fractions, significantly ($P < 0.05$) increased the second predominant methongen genus *Methanomicrobium*. Condensed tannin fractions of different MWs from

* Corresponding author at: Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, UPM Serdang, 43400 Selangor, Malaysia.
E-mail address: norhani@upm.edu.my (N. Abdullah).

LLR could alter the size of populations and diversity of rumen methanogenic archaea *in vitro*, which could contribute to the reduction of CH₄ production, and the impact was more pronounced for CT fractions with higher-MWs.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

On a global scale, enteric methane (CH₄) production from ruminating animals accounts for 8% of total anthropogenic greenhouse gas (GHG) emission, which significantly affects global warming and climate change (Smith et al., 2007; Beauchemin et al., 2008). Methane has a heat trapping potential 34 times that of CO₂ (IPCC, 2013). Moreover, during its lifetime, CH₄ can re-emit and absorb infrared radiation, thereby contributing to global warming. Methane production by ruminating animals also results in a loss of feed energy of up to 12% (Johnson and Johnson, 1995). For these reasons, there are various research initiatives underway to develop CH₄ mitigation strategies for ruminants (McAllister et al., 1996; Martin et al., 2010). However, for cattle, dietary strategies may be the most beneficial as they can be easily implemented. Enteric CH₄ emission reduction in livestock would not only be beneficial for climate control, but also enhance productivity.

Methanogenesis activity in ruminants is associated with the community structure of methanogens. Methanogenic archaea produce CH₄ as an anaerobic fermentation end-product in the rumen during H₂ disposal produced in metabolic activity (Stewart et al., 1997). Methanogens belong to the *Euryarchaeota* phylum and are divided into six orders: *Methanomicrobiales*, *Methanopyrales*, *Methanococcales*, *Methanobacteriales*, *Methanocellales* and *Methanosarcinales* (Paul et al., 2012). Members of these orders are hydrogenotrophic, utilizing H₂/methanol or H₂/CO₂ produced from fiber degradation by rumen microbes. Recently, a new order, *Methanoplasmatales* or *Thermoplasmatales*-related archaea, has been recognized as the seventh order of methanogens (Paul et al., 2012). Members of this new order are methylotrophic, utilizing methylamine (Poulsen et al., 2013). Methanogens are abundant in the rumen and are estimated to be about 10⁷–10⁹ cells/g of rumen content in concentrates-fed ruminants (Joblin, 2005). A variety of rumen methanogen species have been identified by studying small subunit rRNA (ssrRNA) genes from ruminants (Janssen and Kirs, 2008).

Targeting ruminal methanogenic archaea may provide a long-term solution for CH₄ mitigation (Kumar et al., 2009; Hook et al., 2010). Studies have found dramatic shifts in methanogenic populations associated with dietary variations (Wright et al., 2008) and host traits (Zhou et al., 2009). Plant extracts have been used as feed ingredients to mitigate enteric ruminal CH₄ emissions (Waghorn et al., 2002). Methane mitigation through the inclusion of compounds that exhibit methanogen toxicity, such as saponin (Hess et al., 2003) and monensin (Hook et al., 2009), in the ruminant diet has been shown to induce variations in the community structures of methanogens. Condensed tannin (CT)-rich plants such as forages and legumes showed toxic effects on methanogens, resulting in less CH₄ formation in ruminants (Woodward et al., 2001; Animut et al., 2008). A number of studies have demonstrated that mitigation of CH₄ production by CTs both *in vitro* and *in vivo* is related to decrease of methanogenic archaea communities (Tavendale et al., 2005; Bhatta et al., 2009; Tan et al., 2011a; Tan et al., 2011b; Cieslak et al., 2012). Several experiments have also shown that CTs decrease the relative gene expression of ruminal methanogens and change the structure of the methanogenic community (Hook et al., 2010; Longo et al., 2013).

CTs are heterologous compounds comprising complex polymers with flavan-3-ol (catechin) or flavan-3,4-diol (epigallocatechin or delphinidin) subunits linked by interflavan bonds. Molecular weights (MWs) of CTs range from 500 to 20,000 Da (Aerts et al., 1999), while the degree of polymerization (DP) of CTs varies from di-, tri- and tetraflavonoids to higher oligomers (Tan et al., 2011a). Differences in their MWs, chemical structures, and biochemical properties contribute to the differing biological activities and their effects on ruminal digestion (Patra and Saxena, 2009).

Tropical forage legumes, such as *Leucaena*, are used as a feed supplement for cattle because of their high protein content. In Malaysia, the *Leucaena leucocephala* hybrid-Rendang (LLR) has higher CT content than the parent *L. leucocephala*. Unfractionated CTs (FO) from LLR have been found to mitigate enteric ruminal CH₄ emissions, concomitant with shifts in the populations and diversity of bovine rumen methanogens (Tan et al., 2011a; Tan et al., 2011b). Recently we have fractionated CTs FO (MW = 1293.0 Da) to five fractions (F1–F5) using size exclusion chromatography (Saminathan et al., 2014). Fraction F1, which was eluted first, has the highest MW (1265.8 Da), followed by fractions F2, F3, F4 (1028.6, 652.2 and 562.2 Da, respectively) and F5 which has the lowest MW (469.6 Da). Later, we found that the *in vitro* CH₄ gas production decreased significantly with increasing MWs of the CT fractions (F1–F5), with no substantial reduction on dry matter (DM) degradability (Saminathan et al., 2015). However, the effects of CT fractions of different MWs from LLR on rumen methanogenic archaeal population and diversity have not been studied. Thus, the present study was carried out to determine the effects of CT fractions of different MWs on the population and diversity of bovine rumen archaea *in vitro* using real-time PCR and Illumina MiSeq sequencing approaches. To the best of our knowledge, this is the first report describing the use of a barcoded Illumina MiSeq genome sequencer to study the population and diversity of rumen archaea.

Download English Version:

<https://daneshyari.com/en/article/8491244>

Download Persian Version:

<https://daneshyari.com/article/8491244>

[Daneshyari.com](https://daneshyari.com)