

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

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 methanogen genus Methanomicrobium. Condensed tannin fractions of different MWs from 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.