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Inhibition of methane production by the palm oil industrial waste phospholine gum in a mimic enteric fermentation

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ABSTRACT

The potential utilization of phospholine gum, a by-product of the palm oil industry was evaluated using waste sewage sludge (WSS) as a substrate as well as a microbial source to mimic methane production by enteric fermentation. Ruminant animals release enteric methane through their digestive process. The enteric methane is one of the greenhouse gases that can contribute to global warming and should be prevented. In this study, methane production was remarkably inhibited by adding phospholine gum to WSS, even at a low concentration. Phospholine gum reduced the activity of methanogens and *Lactobacillus* sp. and *Megasphaera* sp. which are known as important ruminal microorganisms were detected as bacterial species induced by the addition of phospholine gum to WSS. Also, the addition of phospholine gum triggered an increase in protein concentrations as well as protease activities and stimulated to produce protease and cellulase by which phospholine gum may be degraded. Furthermore, a significant amount of propionate was produced in the presence of phospholine gum. Thus, phospholine gum inhibits methane production without inhibiting the stages of hydrolysis and acidogenesis/acetogenesis. Finally, methane fermentation using the rumen derived from a goat was also inhibited by phospholine gum. Therefore, these results indicate that the phospholine gum has great potential to inhibit methane production as a feed additive for ruminant animals.

1. Introduction

Methane is an extremely potent greenhouse gas because it can trap heat and make the planet warmer. Natural gas, petroleum, agricultural industries such as livestock production, landfills, wastewater treatment, and other industrial processes are sources of anthropogenic methane. Moreover, livestock production accounts for 25% of the anthropogenic methane released from enteric fermentation, particularly from ruminant animals (Lesschen et al., 2011).

Anaerobic digestion is a natural process wherein bacteria convert organic materials into biogases through hydrolytic, acidogenic, acetogenic, and methanogenic reactions (Hassan et al., 2005). Enteric methane production in the rumen undergoes a similar anaerobic digestion process (Bayané and Guiot, 2011; Knapp et al., 2014; Yue et al., 2012). Because the gas produced from fermentation is mainly methane, carbon sources can be utilized for the nutrition and milk production of ruminant animals rather than for methane production. Thus, increases in the quantity and quality of livestock may be achieved if methane production derived from ruminant animals is inhibited (Beauchemin et al., 2008).

As the ruminant animals should utilize nutrients for anabolism rather than methane production, it is important to develop a strategy to mitigate enteric methane emission, which benefits ruminant animals and the environment. To date, many mitigation strategies have been developed such as dietary manipulation, utilization of additives in feed, and administration of vaccines

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(Huhtanen et al., 2015). Feed additives have been recently demonstrated to be the simplest approach to mitigate methane emission. Hristov et al. (2013) found that synthetic additives can inhibit enzymes involved in methane production. Moreover, nitrate additives added to the ruminant's feed exhibit toxicity (Lee and Beauchemin, 2014). The utilization of plant-derived additives in feed is preferable because they are derived from natural sources. However, Wischer et al. (2014) found that chestnut and valonea extracts contribute to the high total cost of the feed. Therefore, it is important to find an alternative natural material that is renewable, abundant, and contributes to low or zero cost of feed production to mitigate methane emission from livestock.

Palm oil production is a major industry in Malaysia and Indonesia. The final stage produces refined palm oil from crude oil through the refining process by removing some undesired constituents. For example, phosphatides, also called as phospholipids or phospholine gums, are constituents of palm oil, which need to be removed by a degumming step. At the end of the degumming process, the gums are collected as a by-product (Haslenda and Jamaludin, 2011). However, until now, no study has described the potential utilization of phospholine gum in any field. However, there are several studies demonstrating that a naturally occurring phospholipids compound produced from soy oil refining called soy lecithin is used as a commercial product in the food industry as an emulsifier, a dispersing agent, or a viscosity regulator (Comas et al., 2006; Ozturk and McClements, 2016) and also in the pharmaceutical industry as skin penetration enhancers, moisturizers and anti-irritants (Mehta and Jindal, 2013; Mouri et al., 2015; Valenta et al., 2000). This by-product is a renewable resource that is potentially useful as a feed additive for ruminant animals.

In this study, WSS that is rich in microbial community is used as a substrate for methane production to evaluate the effect of phospholine gum and the dynamics of microbial community. WSS was also used as microbial sources which may simulate the rumen digestion in the ruminant animals. The effect of phospholine gum was also tested by using the rumen derived from a goat.

2. Materials and methods

2.1. Phospholine gum

Phospholine gum (a waste produced from a process of crude palm oil refining) was kindly obtained from Sinarmas Palm Oil Company, Medan, Indonesia. The phospholine gum was directly used for methane fermentation.

2.2. Preparation of sewage sludge

WSS was acquired from the Hiagari Wastewater Treatment Plant in Kitakyushu City, Japan. Washing steps were performed for the WSS by the centrifugation at 8000 × g for 10 min at 4 °C to remove the initial supernatant containing endogenous compounds The supernatant was discarded and the remaining pellet was resuspended in distilled water by vigorous shaking. These steps were repeated for three times. Finally, the pellet was resuspended in distilled water and adjusted to the final concentration of 10% (wet sludge [w/w]).

2.3. Methane assay

Anaerobic digestion was performed using 66-mL vials to fundamentally comprehend the role of phospholine gum for methane production and microbial activity in WSS since WSS contains a variety of microbes. WSS and different concentrations of phospholine gum were placed into 66-mL vials to make 30 mL of the total volume. The vials were tightly sealed using butyl rubber stoppers, crimped, and sparged with nitrogen gas for 2 min to create anaerobic conditions. The vials were then incubated at 37 °C and shaken at 120 revolutions per minute (rpm) for 10 days. The control vial was prepared using the same method but without phospholine gum. Each experiment was conducted at least in triplicate. Methane was measured by injecting 50 μL of headspace gas from the vials into a GC-3200 gas chromatograph (GL Science, Japan) equipped with a thermal conductivity detector and a column of Molecular Sieve 13 × 60/80 mesh column, SUS 2 × 3 mm I.D (GL Science, Japan). Helium gas was used as a carrier gas (40 mL/min). The gas chromatography conditions were as follows: current, 100 mA; oven, injector, and detector temperatures, 40 °C, 50 °C, and 65 °C, respectively.

2.4. Analytical methods

WSS was sampled during the fermentation for the following analyses: organic acids, pH, protein concentration, and protease activity. Initially, the fermented WSS samples were centrifuged at 13,000 rpm for 7 min to collect the supernatants which were then filtered through a 0.2-μm membrane syringe filter. Organic acids were analyzed using high-performance liquid chromatography (Shimadzu LC-10AD) as described previously (Mohd Yusoff et al., 2012). The pH was measured using a compact pH meter (AS ONE, AS-211, Japan). The soluble protein concentration was analyzed using the Lowry method with bovine serum albumin as a standard (Lowry et al., 1951). Protease activity was measured as described previously (Maeda et al., 2011). One unit of protease activity was defined as the quantity of tyrosine (μmol) produced from casein per minute by 1 mg of enzyme. Each assay was conducted at least in triplicate.

2.5. RNA extraction and cDNA synthesis

Ribonucleic acid (RNA) was extracted from raw WSS and fermented WSS. Before RNA extraction, 3 mL of RNAlater solution (Ambion, Cat#AM7020) was mixed with 4 mL of a WSS sample to avoid RNA degradation. The mixture was then centrifuged at 15,000 rpm for 2 min at 4 °C to harvest the sludge pellet. The pellet was re-suspended in 1 mL RNAlater solution and centrifuged at 13,000 rpm for 30 s. After removing the supernatant, the remaining sludge pellet was quickly frozen in a dry ice-ethanol bath and stored at −70 °C. Total RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA) as described previously (Mohd Yusoff et al., 2012). The concentration of RNA extracted was measured using a NanoDrop spectrophotometer (SCRUM Inc., Japan). The complementary deoxyribonucleic acid (cDNA) was synthesized using the PrimeScript RT Reagent Kits (TAKARA Bio Inc., Shiga, Japan). The synthesis was performed using 5 μg of total RNA in a 10 μL mixture containing 2 μL of 5 × PrimeScript buffer, 0.5 μL reverse transcriptase, 0.5 μL oligo(dT) primer, and 2 μL random oligomers. The mixture was incubated at 25 °C for 10 min followed by 37 °C for 30 min, and the enzyme was inactivated at 85 °C for 5 s. Electrophoresis was performed to determine the quality and quantity of the RNA and cDNA. The cDNA was used as a template to identify bacterial and archaeal population and microbial communities using quantitative real-time polymerase chain reaction (qRT-PCR) and MiSeq, respectively.

2.6. qRT-PCR

A TaqMan system was used to perform qRT-PCR to quantify 16S ribosomal RNAs (rRNA) of bacteria and archaea. The StepOne Real Time PCR System (Applied Biosystem) was used for amplification