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Propionic acid bacteria enhance ruminal feed degradation and reduce methane production *in vitro*

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ABSTRACT

Thirty-one strains of propionic acid bacteria were screened for their effects on methane production and volatile fatty acid concentrations using *in vitro* assays of rumen fluid from Norwegian dairy cows and a grass silage–concentrate mixture as substrate. Nine of 31 strains were further analysed for effects on substrate degradation. Propionic acid bacteria led to reductions of up to 20% in methane production. Seven strains stimulated volatile fatty acid production, and in their presence *in vitro* substrate degradation tended to increase ($P < .10$). Most consistent results were found with *Propionibacterium thoenii* T159, which reduced methane production by 20% and caused 8% and 21% overall increases in substrate degradation and total volatile acid production, respectively ($P < .05$). Concomitant beneficial effects of a reduction in methane emissions and an increase in feed degradation suggest that this strain may be a promising tool for improving the productive performance of dairy cows.

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Propionibacterium; methane mitigation; rumen fluid; dairy cow; Norwegian Red; volatile fatty acids

Introduction

The rumen microbiome, which ferments the feed and thereby provides fermentation end products that can be utilized by the host animal, is crucial in ruminant digestion. Accordingly, measures that aim at improving feed utilization and animal productivity by feeding live microbes to ruminants has a long tradition (Nocek & Kautz, 2006; McAllister et al., 2011). A major goal is to reduce enteric methane formation during rumen fermentation. Methane production represents an energy loss for the ruminant constituting 3 to 10% of its gross-energy intake (Niu et al. 2018). Moreover, methane is an important greenhouse gas and about 17% of all anthropogenic methane released into the atmosphere originates from domestic ruminants (Knapp et al. 2014). Methane is produced by methanogens from CO₂ and hydrogen formed during the microbial fermentation of the feed into volatile fatty acids (VFA) (Boadi et al., 2004). Acetate, propionate and butyrate are the major VFA produced that enter the bloodstream and serve as a source of energy and as a substrate for anabolic functions in ruminants. The propionate fermentation pathway is distinguished from the pathways leading to acetate and butyrate by not liberating hydrogen (Boadi et al., 2004). Hence, a positive correlation between

enteric methane production and the ratio of ruminal acetate to propionate has been established (Russell, 1998). Therefore, stimulating propionate fermentation in ruminants might lower methane emissions. Moreover, unlike acetate and butyrate, propionate is a gluconeogenic VFA and thus can increase the availability of energy to the mammary gland (Yost & Young, 1977; Zárate, 2012). Propionate is an end product of the fermentation of various bacterial species, including the organisms of the family *Propionibacteriaceae*. Fed to dairy cows, strains of *propionic acid bacteria* (PAB) have been reported to have positive effects on production, including increased propionate levels in the rumen and improved milk yield (Stein et al., 2006; Adams et al. 2008; de Ondarza & Seymour, 2008; Weiss et al., 2008). Vyas et al. (2014) and Jeyanathan et al. (2019) studied the effects of *Propionibacterium* on methane production in ruminants. None of the strains tested was found to affect the total methane production. However, Vyas et al. (2014) observed that the feed intake was higher in the beef heifers fed *Propionibacterium* and the ratio between the methane produced and the feed consumed was lower by 8% to 13%. Other PAB strains were studied using *in vitro* experiments on rumen fluid from Canadian beef cattle (Alazeh et al., 2013). That work showed that

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three PAB strains could reduce methane formation *in vitro* by 7 to 15%, yielding decreased proportions of acetate, and/or increased propionate proportions or yielding no change in the proportion of these VFA.

In this work, using rumen fluid from Norwegian Red dairy cows, we studied a large number of PAB isolates for their ability to affect the production of methane and of VFA and the ruminal feed degradation *in vitro*.

Materials and methods

Bacterial strains and growth conditions

To search for strains that could favourably alter rumen metabolism, we initially screened a total of 149 PAB strains for their effects on methane and gas production *in vitro* (data not shown). Most of the strains from the initial screening had no effect on methane production. Thirty-one of the 149 PAB strains chosen for the present study (28 strains of the genus *Propionibacterium*, 2 strains of *Tessaracoccus* and 1 strain of *Luteococcus*, Tables 2 and 3) all showed inhibitory effects on methane production. We conducted additional *in vitro* experiments with 9 promising strains out of the 31, in which feed degradation was measured as the weight difference before and after incubation in the rumen fluid. The PAB strains used in this study were isolated from Norwegian Red raw milk. These strains have been shown to produce propionic acid from lactic acid under anaerobic conditions (Holo et al., 2002). The strains were grown anaerobically at 37 °C in 10 mL screw capped glass tubes filled with sodium lactate broth (SLB) containing 10% tryptone, 10% yeast extract, 1.2% sodium lactate and 0.25 g of K₂HPO₄ per litre (Malik et al., 1968). Cultures to be used in the *in vitro* experiments were inoculated with 100 µL of exponentially growing cells and incubated for two days before used in the experiments. Viable counts of PAB were determined after anaerobic incubation at 37 °C on solidified SLB that contained 1.5% agar. For the determination of viable PAB in samples from the *in vitro* experiments, the agar plates were supplemented with metronidazole (4 µg/mL) (Jan et al., 2002).

Feed and rumen fluid

A feed mixture whose composition was comparable to that used for feeding Norwegian Red dairy cows in peak lactation was used as the substrate for the *in vitro* incubations (Table 1). The feed dry matter (DM) used in the *in vitro* experiments contained 60% grass silage and 40% concentrate milled to pass 4 mm and 1 mm screens, respectively. The concentrate DM contained 40% barley, 40% wheat and 20% soybean meal.

Table 1. Ingredients and chemical composition of the substrate (% dry matter, DM) used in the *in vitro* experiments 1 to 6.

Item	% DM
Ingredients	
Grass silage	60
Barley	16
Wheat	16
Soybean meal	8
Chemical composition	
Crude protein	16.5
Acid detergent fibre	27.1
Neutral detergent fibre	43.4
Non-fibrous carbohydrates	33.8

Rumen fluid was obtained from two non-lactating Norwegian Red cows, both fitted with a permanent ruminal cannula. The cows were kept in a metabolism unit authorized by the Norwegian Animal Research Authority and fed a standardized diet consisting of grass and concentrate that met maintenance requirement. The concentrate mixture contained approximately 180 g crude protein (CP)/kg DM and 120 g neutral detergent fibre (NDF)/kg DM. Rations of hay and concentrate were offered in equal meals at 06.30 h and 14.30 h. Samples of rumen fluid from the two cows were taken through the fistulae two hours after morning feeding, filtered through two layers of cheese cloth, mixed in a ratio of 1:1, flushed with CO₂ and then, within 30 minutes, mixed with two parts of the buffer prepared according to Menke et al. (1979). This freshly prepared buffered rumen fluid was used for the *in vitro* experiments.

In vitro experiments and sampling

During initial screening and in subsequent experiments 1 and 2, 10 mL of buffered rumen fluid (Menke et al., 1979) was mixed with 100 µL of the PAB culture (2×10^8 to 4×10^8 colony forming units, CFU, in total) and 50 mg of feed in 25 mL serum bottles under a stream of CO₂. The bottles were sealed with rubber serum stoppers and aluminium crimp caps and incubated for 24 h at 39°C. The control samples were supplemented with 100 µL of sterile SLB instead of PAB culture. Experiments 3, 4, 5 and 6 were conducted as described above, except that the incubations were carried out in stoppered 125 mL serum bottles using 450 µL of the PAB culture (or 450 µL SLB), 45 mL of buffered rumen fluid and 500 mg of feed. During screening and in experiments 3 to 6, all treatments and controls were carried out in triplicate. In experiments 1 and 2, treatments were carried out in triplicate and control samples were replicated 9 times.

The pressure in the vials was measured after 24 h of incubation using a DPG-200 Digital Pressure Gauge (Dwyer Instruments, Inc., Michigan City, IN). Gas samples were taken from the headspace of the vials

with a gas tight syringe and the methane concentration was measured using a gas chromatograph (Model 7890A, Agilent, Santa Clara, CA, US) that had a 20-m wide-bore Poraplot Q (0.53 mm) column at 38°C with back flushing and He as the carrier gas. The methane production (V_{CH_4} , mL) was calculated as:

$$V_{\text{CH}_4} = \text{ppm}_{\text{CH}_4} \times V_{\text{HS}} \times 10^{-6} \times \Delta P$$

where V_{HS} is the volume of the vial headspace (mL) and ΔP is the vial pressure relative to standard pressure (atm).

Liquid samples for the analyses of the VFA concentrations were stored at -20°C. To determine the feed degradation in the 9 strains in experiments 3, 4, 5 and 6, the contents of the rinsed bottles were centrifuged at 7000 × g for 10 min in 50 mL of pre-weighed polypropylene centrifuge tubes. The supernatants were discarded, and the pellets were washed twice with distilled water and oven dried at 65°C for 7 days and weighed. The amount of feed DM that had been digested was calculated as the difference in weight between the pellets from samples taken at the start and the end of the incubation.

Chemical analysis

The contents of CP, acid detergent fibre (ADF), NDF and non-fibrous carbohydrates (NFC) in mixed feed DM were determined by Dairy One Forage Laboratory (Ithaca, NY) using near-infrared reflectance spectroscopy (Table 1).

The methane concentrations of the gas samples were determined using a flame ionization detector calibrated to certified standards of 2, 100 and 10,000 ppmv (Yara, Norway).

For the VFA analysis, liquid samples were thawed and 5 mL aliquots were mixed with 0.3 mL of 50% H₂SO₄ and 15 g of sodium sulphate and extracted with 25 mL of diethyl ether. Samples from the ether phase were analysed for VFA by gas chromatography using a Perkin Elmer Autosystem GC equipped with a flame ionization detector and a Supelco 2 m, 0.635 cm OD and a 2 mm ID glass column, packed with GP 10% SP 1000/1% H₃PO₄ on a 100/120 Chromosorb WAW (Kraggerud et al., 2014). The injection temperature was 210°C and the carrier gas was nitrogen at 40 mL/min. The following temperature programme was applied: 120°C for 1 min and then 15°C/min to 190°C for 5 min.

Statistical analysis

The results were analysed using a one-way analysis of variance (ANOVA), with the strain as the fixed factor and the vial as the random factor. Then Fisher's least significant difference (LSD) post hoc test was conducted using a 95% confidence interval (Minitab version 19 from Minitab Inc., PA, USA).

Results and discussion

Effects on production of volatile fatty acids and methane

The control samples in experiments 1 and 2 produced 48 mL and 51 mL methane per g of feed DM, respectively (Tables 2 and 3). Most of the PAB strains tested did not cause a significant change in methane production, which ranged between 42 and 54 mL/g of feed DM. However, incubation with *P. jensenii* LMGT2826 and

Table 2. Effect of propionic acid bacterial strains *Propionibacterium* (*P.*) and *Tessaracoccus* (*T.*) on production of methane and volatile fatty acids in experiment 1.

Bacterium added	Strain	Total VFA ^a , mM	Acetate, mM/ 100 mM total VFA	Propionate, mM/ 100 mM total VFA	Butyrate, mM/ 100 mM total VFA	Acetate: propionate	Methane, mL/g DM
<i>P. sp.</i>	LMGT2789	77.0	68.9	16.8	14.2	4.09	48.1
<i>P. acidipropionicii</i>	LMGT2831	75.4	68.6	17.2	14.2	4.00	49.7
<i>P. freudenreichii</i>	LMGT2832	76.1	67.9	18.0*	14.1	3.78*	43.9
<i>P. freudenreichii</i>	LMGT2833	77.1	68.7	17.1	14.2	4.02	47.1
<i>P. freudenreichii</i>	LMGT2842	72.4	67.6*	17.6	14.7	3.84	55.0
<i>P. jensenii</i>	LMGT2864	71.7	67.6*	17.3	15.1*	3.90	47.3
<i>P. sp.</i>	T1	76.2	68.6	17.1	14.3	4.01	47.5
<i>P. sp.</i>	T25	78.7	69.3	17.1	13.7	4.06	49.0
<i>P. freudenreichii</i>	T28	80.4*	69.6	16.9	13.5	4.12	50.1
<i>P. freudenreichii</i>	T30	73.0	67.9	16.8	15.3*	4.05	47.1
<i>P. freudenreichii</i>	T62	79.6*	69.1	16.9	14.0	4.08	47.8
<i>P. sp.</i>	T88	81.2*	69.8*	17.0	13.2*	4.11	52.1
<i>T. bendingoniensis</i>	T93	71.9	67.5*	17.6	14.9	3.83	48.4
<i>T. bendingoniensis</i>	T104	77.2	69.3	17.0	13.7	4.09	48.6
<i>P. freudenreichii</i>	T114	82.0*	69.4	17.1	13.5	4.06	53.7
<i>P. acidipropionicii</i>	T122	77.2	69.2	17.3	13.5	4.00	45.2
None	Control	74.1	68.7	17.1	14.2	4.01	47.9
	SEM	2.01	0.36	0.24	0.35	0.06	0.68

Mean values with an asterisk differ significantly ($P < .05$) from control.

^aSum of acetate, propionate and butyrate.

Table 3. Effect of propionic acid bacterial strains *Propionibacterium* (*P.*) and *Luteococcus* (*L.*) on production of methane and volatile fatty acids in experiment 2.

Bacterium added	Strain	Total VFA ^a , mM	Acetate, mM/100 mM total VFA	Propionate, mM/100 mM total VFA	Butyrate, mM/100 mM total VFA	Acetate: propionate	Methane, mL/g DM
<i>P. jensenii</i>	LMGT2816	79.4	72.8	15.9	11.3*	4.57	46.6
<i>P. jensenii</i>	LMGT2822	73.6	72.5	16.0	11.5	4.52	44.6
<i>P. jensenii</i>	LMGT2823	73.4	71.8	16.2	12.1	4.44	46.8
<i>P. jensenii</i>	LMGT2824	73.8	71.6	16.2	12.2	4.41	49.7
<i>P. jensenii</i>	LMGT2825	71.3	71.2	16.4	12.4	4.35	47.9
<i>P. jensenii</i>	LMGT2826	75.5	72.4	16.3	11.4*	4.45	41.7*
<i>P. thoenii</i>	LMGT2827	74.1	71.5	16.2	12.2	4.41	47.0*
<i>P. freudenreichii</i>	LMGT2841	75.0	72.2	16.1	11.7	4.49	47.3
<i>P. sp.</i>	T22	71.4	71.1	16.4*	12.4	4.33*	47.4
<i>P. sp.</i>	T24	85.6*	71.9	16.1	12.0	4.51	43.3
<i>P. freudenreichii</i>	T27	73.1	72.2	16.0	11.9	4.52	45.3
<i>P. freudenreichii</i>	T31	81.4	72.9	15.9	11.2*	4.57	44.5
<i>P. propionicus</i>	T83	85.9*	74.1*	15.2	10.7*	4.88*	43.9
<i>P. sp.</i>	T88	80.3	72.4	15.8	11.8	4.58	41.7
<i>L. japonicus</i>	T145	75.8	72.4	15.9	11.7	4.54	47.8
<i>P. thoenii</i>	T159	89.6*	72.5	16.7*	10.8*	4.34	40.7*
None	Control	74.0	72.0	15.8	12.2	4.57	51.0
	SEM	2.53	0.37	0.22	0.25	0.08	0.42

Mean values with an asterisk differ significantly ($P < .05$) from control.

^aSum of acetate, propionate and butyrate.

P. thoenii LMGT2827 or T159 resulted in reductions ($P < .048$) in methane production of 18%, 8% and 20%, respectively, relative to the control samples. In previous reports, the effects of *Propionibacterium* had been found in experiments with dairy cattle, in particular using the *Propionibacterium acidipropionici* strain P169, which may increase the efficiency of milk production (de Ondarza & Seymour, 2008; Weiss et al., 2008), but other strains tested did not show this effect (Seo et al., 2010). Using rumen fluid from cattle, it was shown that several strains of PAB could reduce ruminal methane production *in vitro* (Alazzeah et al., 2013), but this effect was not seen with strain P169 (Alazzeah et al., 2014). This finding conflicts with that of Stein et al. (2006); they found that the strain P169 caused higher rumen propionate levels *in vivo*.

The total VFA concentrations (the sum of acetate, propionate and butyrate concentrations) in the controls at 74 mM were very similar in experiments 1 and 2 (Tables 2 and 3). None of the PAB strains caused a significant reduction in total VFA production, but seven strains had stimulatory effects ($P \leq .046$). Relative to the controls, the strain T159 showed the strongest stimulation of VFA production, increasing levels by 21% ($P = .001$), with the proportion of propionate being higher by 6%, but without a change in acetate. The presence of four (T28, T62, T114 or T24) of the seven stimulatory strains did not change the proportions of acetate, propionate and butyrate. With the addition of the strain T83, the proportion of acetate increased slightly compared to the controls ($P < .001$), leading to a higher ratio of acetate to propionate ($P = .011$). The strain T88 was tested in both experiments and it increased ($P \leq .024$) VFA production by about 10%

and the proportion of acetate by 2% in experiment 1 (Table 2) but did not do so in experiment 2 (Table 3), relative to the controls. The majority of the 31 strains had no effect on the proportions of butyrate; only T88, T83 and T159 led to a slight decrease in proportion of butyrate compared to that in the controls ($P \leq .038$).

While the dominating VFA in the rumen is acetate, the metabolic end products of the PAB that grow on sugars or lactate are propionate and acetate in a ratio of 2:1 or higher. Previous studies found that PAB strains could increase rumen propionate levels (as a proportion of the total VFA) and lower levels of acetate *in vivo* (Stein et al., 2006; Raeth-Knight et al., 2007; Weiss et al., 2008) and *in vitro* (Akay & Dado, 2001; Alazzeah et al., 2013), suggesting that PAB could significantly contribute to rumen fermentation. Luo et al. (2017) showed that PAB can degrade lactate in the rumen fluid *in vitro*. In experiments 1 and 2, the molar ratios of acetate to propionate in the controls were 4.0 and 4.6, respectively. Slightly lowered acetate to propionate ratios ($P \leq .048$) were observed using the strains *P. freudenreichii* LMGT2832 and *P. sp.* T22 than in the controls.

Including metronidazole in the SLB agar plates (Jan et al., 2002) enabled us to study the fate of the PAB after 24 h of incubation. With no PAB in the sample, we found a viable count of 1×10^6 CFU per mL on this medium in the buffered rumen fluid, and this number increased about two-fold over 24 h in the control vials without PAB (data not shown). None of the PAB strains added to the vials exhibited growth in the bottles during incubation, and their viable count decreased by 17% (the strain LMGT2826) to 95% (the strain LMGT2827) (results not shown). Although we observed a

reduction in the number of PAB cells during incubation, we cannot rule out the initial growth of the PAB followed by a death phase during the 24 h of incubation. However, PAB grow slowly; using published data on the maximal specific propionate production and the growth rate (Lee et al., 1974), we calculated that the average rate of propionate synthesis in our experiments would be lower than the observed stimulation, compared to the controls.

Thus, most of the propionate in our experiments must have been produced by other organisms in the rumen samples. For the same reason, it seems unlikely that the increase in propionate observed when feeding P169 is produced by the strain P169 itself, since the recovery for this strain was 10^6 CFU per mL or lower (Peng et al., 2011). However, Luo et al. (2017) showed that propionate can be formed by PAB at high rates and we cannot rule out that P169 could have produced propionate more rapidly *in vivo* than *in vitro*, as a result of the stimulation of natural microbial inhabitants of the rumen.

Our results with rumen liquid from Norwegian Red dairy cattle differ somewhat from those in previous reports with respect to ruminal VFA proportions. Two strains led to an increase in acetate proportions but not to a concomitant increase in methane yield. Three other strains tested in the present study caused increases in the propionate but no change in the acetate proportions or the methane yield, except for *P. thoenii* T159, which led to a 20% reduction in the methane yield. This shows that the PAB strains *in vitro* were able to partially redirect the carbon flow. Moreover, the PAB strains LMGT2826, LMGT2827 and T159 were even able to redirect it away from methanogenesis.

The data indicate that although propionate fermentation is stimulated, acetogenesis, possibly homoacetogenesis (Joblin, 1999), appears to be stimulated even more in some cases. Methane production in our experiments was 40.7 to 55.0 mL/g DM, a little higher than reported for Canadian cattle (Alazeh et al., 2013;

Alazeh et al., 2014). In our study, the methane production corresponded to about 0.1 mmol C in the methane per vial. At most, this was reduced compared to the controls by 0.02 mmol C (the strain T159), while the observed increase in VFA production with this strain corresponded to 0.35 mmol C. Thus, our results cannot be explained merely as the redirection of the carbon flow away from methanogenesis to VFA production. Rather, the data indicate increased feed degradation and fermentation through pathways that produce mainly acetate and, to a lesser degree, propionate. They also indicate that PAB strains can stimulate metabolism of other microbes in the rumen.

The PAB have been shown to affect the growth of a wide variety of microbes, including bacteria, yeasts and moulds. Most studies have focused on selectively inhibiting the growth of microbes (Holo et al., 2002; Lind et al., 2007; Schwenninger et al., 2008; Faye et al., 2011), but the PAB's properties of stimulating growth are also known (Kaneko, 1999; Jan et al., 2002; Warminska-Radyko et al., 2002); 1,4-dihydroxy-2-naphthoic acid secreted by PAB can stimulate the growth of *Bifidobacteria* and various other anaerobes (Kaneko, 1999; Fenn et al., 2017). In line with this, it was reported that *P. acidipropionicii* P169 could stimulate rumen feed degradation (Sanchez et al., 2014).

Feed degradation

In the controls, degradation, that is, the amount of substrate digested after 24 h ranged from 57% to 63% (Table 4). The DM degradation varied greatly when the PAB strains were added, except for the strains LMGT2825 and LMGT2841. With these two particular strains, we saw no stimulation of degradation. Each of the other strains increased feed degradation ($P < .05$) in at least one of the four experiments. In experiment 5, several strains increased degradation to about 72%.

Table 4. Effect of propionic acid bacterial strains *Propionibacterium* (*P.*) and *Tessaracoccus* (*T.*) on substrate degradation in experiments (Exp.) 3 to 6.

Bacterium added	Strain	Substrate degradation (% dry matter)				Average
		Exp. 3	Exp. 4	Exp. 5	Exp. 6	
<i>P. jensenii</i>	LMGT2816	60.9	59.2	64.4	71.3*	63.9
<i>P. jensenii</i>	LMGT2824	54.6*	60.2	57.0	69.7*	60.4
<i>P. jensenii</i>	LMGT2825	59.6	59.6	60.5	57.8	59.4
<i>P. freudenreichii</i>	LMGT2841	59.0	60.6	63.5	60.5	60.9
<i>P. sp.</i>	T24	58.5	60.5	72.3*	60.4	63.0
<i>P. freudenreichii</i>	T31	58.9	59.5	72.4*	62.6	63.3
<i>P. sp.</i>	T88	61.3	58.6	71.3*	63.2	63.6
<i>T. bendingoniensis</i>	T93	61.1	59.2	69.2*	64.7	63.5
<i>P. thoenii</i>	T159	57.2	63.3*	71.7*	67.5	64.9*
None	Control	56.5	59.4	61.0	63.2	60.0
	SEM	0.55	0.32	1.11	0.82	1.97

Mean values within a column with an asterisk differ significantly ($P < .05$) from control.

Only one strain, LMGT2824 in experiment 3, resulted in a slight (4%) reduction in feed degradation.

The strongest overall stimulation ($P < .008$) was obtained with strain T159, with an average increase in feed degradation of 8% compared to the controls. The strains LMGT2816, T88 and T93 showed the same trend ($P \leq .057$), with an average increase in degradation of 6%. The beneficial effects of PAB on ruminal feed degradation are not well understood. The PAB might stimulate the growth and activities of ruminal microorganisms by providing growth factors, e.g. 1,4-dihydroxy-2-naphthoic acid (Kaneko, 1999; Fenn et al., 2017).

The variability in feed degradation across experiments was greater in incubations with PAB than in the controls, ranging from no effect to 12% increase. This may reflect day-to-day differences in the rumen microbiome or in the condition of the PAB cultures used.

We have found that PAB can increase feed degradation. This may be a general property of PAB, but the strain T159 showed the most consistent results. Interestingly, the same strain also showed the strongest effects in the *in vitro* test with rumen fluid from Canadian beef cattle (Alazzeah et al. 2013). In those experiments, with different feed, the effects on methane yield were smaller, although propionate was stimulated at the expense of acetate. It is noteworthy that the strain T159 influenced the metabolism of rumen microbiota that were likely quite different in our and Alazzeah et al.'s (2013) studies, as evidenced by the differences in the ruminal acetate to propionate ratios. Such differences and the different feeds used may have contributed to the somewhat different outcomes in the two studies.

Using a diet typical for dairy cows, we have shown that *P. thoenii* T159 can stimulate feed degradation and inhibit methane formation at the same time. These two beneficial traits add the strain T159 to the list of promising direct-fed microbials for more efficient feed utilization by ruminants, but this has to be evaluated *in vivo*.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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