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Authors	Doyle, Natasha;Mbandlwa, Philiswa;Kelly, William J.;Attwood, Graeme;Li, Yang;Ross, R. Paul;Stanton, Catherine;Leahy, Sinead
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Use of Lactic Acid Bacteria to Reduce Methane Production in Ruminants, a Critical Review

Natasha Doyle[1,2†], Philiswa Mbandlwa[2†], William J. Kelly[3†], Graeme Attwood[4], Yang Li[4], R. Paul Ross[2,5], Catherine Stanton[1,5] and Sinead Leahy[4]*

[1] Teagasc Moorepark Food Research Centre, Fermoy, Ireland, [2] School of Microbiology, University College Cork, Cork, Ireland, [3] Donvis Ltd., Palmerston North, New Zealand, [4] AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand, [5] APC Microbiome Ireland, University College Cork, Cork, Ireland

Enteric fermentation in ruminants is the single largest anthropogenic source of agricultural methane and has a significant role in global warming. Consequently, innovative solutions to reduce methane emissions from livestock farming are required to ensure future sustainable food production. One possible approach is the use of lactic acid bacteria (LAB), Gram positive bacteria that produce lactic acid as a major end product of carbohydrate fermentation. LAB are natural inhabitants of the intestinal tract of mammals and are among the most important groups of microorganisms used in food fermentations. LAB can be readily isolated from ruminant animals and are currently used on-farm as direct-fed microbials (DFMs) and as silage inoculants. While it has been proposed that LAB can be used to reduce methane production in ruminant livestock, so far research has been limited, and convincing animal data to support the concept are lacking. This review has critically evaluated the current literature and provided a comprehensive analysis and summary of the potential use and mechanisms of LAB as a methane mitigation strategy. It is clear that although there are some promising results, more research is needed to identify whether the use of LAB can be an effective methane mitigation option for ruminant livestock.

Keywords: lactic acid bacteria, methane, methanogens, bacteriocins, direct-fed microbials, silage inoculants, mitigation

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***Correspondence:**
Sinead Leahy
sinead.leahy@agresearch.co.nz

† These authors have contributed equally to this work

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INTRODUCTION

While ruminant animals play an important role in sustainable agricultural systems (Eisler et al., 2014) they are also an important source of greenhouse gas (GHG) emissions (Reisinger and Clark, 2018). Regardless of the ruminant species, the largest source of GHG emissions from ruminant production is methane (CH_4), with more than 90 percent of emissions originating from enteric fermentation (Opio et al., 2013). Enteric fermentation is a digestive process by which a community of microbes present in the forestomach of ruminants (the reticulo-rumen) break down plant material into nutrients that can be used by the animal for the production of high-value proteins that include milk, meat and leather products. Hydrogen (H_2) and methyl-containing compounds generated as fermentation end products of this process are used by different groups of rumen methanogenic archaea to form CH_4, which is belched and exhaled from the lungs via respiration from the animal and released to the atmosphere. In the coming decades, livestock farmers will face

numerous challenges and the development of technologies and practices which support efficient sustainable food production while moderating greenhouse gas emissions are urgently required. More than 100 countries have committed to reducing agricultural GHG emissions in the 2015 Paris Agreement of the United Nations Framework Convention on Climate Change, however, known agricultural practices could deliver just 21–40% of the needed reduction, even if implemented fully at scale (Wollenberg et al., 2016). New technical mitigation options are needed. Reviews of CH_4 mitigation strategies consistently discuss the possibility that lactic acid bacteria (LAB) could be used to modulate rumen microbial communities thus providing a practical and effective on-farm approach to reducing CH_4 emissions from ruminant livestock (Hristov et al., 2013; Takahashi, 2013; Knapp et al., 2014; Jeyanathan et al., 2014; Varnava et al., 2017). This review examines the possible contribution of LAB in the development of an on-farm CH_4 mitigating strategy.

RESULTS AND DISCUSSION

General Characteristics of Lactic Acid Bacteria

Lactic acid bacteria are Gram positive, acid tolerant, facultatively anaerobic bacteria that produce lactic acid as a major end-product of carbohydrate fermentation (Stilez and Holzapfel, 1997). Biochemically they include homofermenters that produce primarily lactic acid, and heterofermenters that also give a variety of other fermentation end-products such as acetic acid, ethanol and CO_2. LAB have long been used as starter cultures for a wide range of dairy, meat and plant fermentations, and this history of use in human and animal foods has resulted in most LAB having Qualified Presumption of Safety (QPS) status in the European Union or Generally Recognized as Safe (GRAS) status in the United States. The main LAB genera used as starter cultures are *Lactobacillus*, *Lactococcus*, *Leuconostoc,* and *Pediococcus* (Bintsis, 2018) together with some species of *Enterococcus* and *Streptococcus*.

In addition to their contribution to the development of food flavor and texture, LAB have an important role in inhibiting the growth of spoilage organisms through the production of inhibitory compounds. These compounds include fermentation products such as organic acids and hydrogen peroxide as well as ribosomally synthesized peptides known as bacteriocins (Cotter et al., 2013). In many cases, the physiological role of bacteriocins is unclear but they are thought to offer the producing organism a competitive advantage, via their ability to inhibit the growth of other microorganisms, particularly in complex microbial communities. Some strains also produce other compounds such as non-ribosomally synthesized peptides which may have additional antimicrobial activity (Mangoni and Shai, 2011).

In recent years much interest has been shown in the use of LAB as probiotic organisms and in their potential contribution to human health and well-being. LAB have also been advocated as probiotics to improve food animal production and as alternatives to antibiotics used as growth promotors (Vieco-Saiz et al., 2019).

LAB and the Rumen

LAB are members of the normal gastrointestinal tract microbiota, however, in ruminants these organisms are generally only prevalent in young animals before the rumen has properly developed (Stewart et al., 1988). LAB are unable to initiate the metabolism of plant structural polysaccharides and are not regarded as major contributors to rumen fermentation. In the Global Rumen Census project (Henderson et al., 2015) which profiled the microbial community of 684 rumen samples collected from a range of ruminant species, only members of the genus *Streptococcus* were found in a majority of samples (63% prevalence, 0.5% abundance). Nevertheless, LAB can be readily isolated from the rumen, with some species such as *Lactobacillus ruminis* and *Streptococcus equinus* (formerly *S. bovis*) being regarded as true rumen inhabitants while others (*Lactobacillus plantarum* and *Lactococcus lactis*) are likely to be transient bacteria that have been introduced with the feed (Stewart, 1992). Several obligately anaerobic rumen bacteria also produce lactate as a fermentation end product and two of these are included in this review. These organisms (*Kandleria vitulina* and *Sharpea azabuensis*) are both members of the family *Erysipelotrichaceae* within the phylum Firmicutes, although *Kandleria vitulina* was formerly known as *Lactobacillus vitulinus* (Salvetti et al., 2011). *Sharpea* and *Kandleria* are a significant component of the rumen microbiome in low CH_4 yield animals in which rapid heterofermentative growth results in lactate production (Kamke et al., 2016).

Table 1 lists the rumen LAB together with strains of *Kandleria* and *Sharpea* that have been genome sequenced along with potential antimicrobial biosynthetic clusters predicted from the genome sequence data. The majority (81%) of genome sequenced strains from rumen members of the *Streptococcaceae* encode antimicrobial biosynthetic clusters, and previous studies have also reported that rumen streptococci can produce a range of bacteriocins (Iverson and Mills, 1976; Mantovani et al., 2001; Whitford et al., 2001). Conversely, antimicrobial biosynthetic genes have not been identified from the species *Kandleria vitulina* and *Sharpea azabuensis*.

How Are LAB Used in Ruminant Agriculture?

On-farm, LAB are used as direct-fed microbials (DFMs), probiotics and as silage inoculants. The terms DFM and probiotic are used interchangeably in animal nutrition and refer to any type of live microbe-based feed additive. Although the products have different purposes, there is considerable overlap in the bacterial species used.

The efficacy of DFMs containing LAB has been studied mostly in pre-ruminants where their reported benefits include a reduction in the incidence of diarrhea, a decrease in fecal shedding of coliforms, promotion of ruminal development, improved feed efficiency, increased body weight gain, and reduction in morbidity (Krehbiel et al., 2003). A meta-analysis of randomized controlled trials of LAB supplementation in young calves has shown that LAB can exert a protective effect and reduce the incidence of diarrhea (Signorini et al., 2012) and can increase

TABLE 1 | List of rumen LAB cultures in addition to a further two species of obligately anaerobic rumen bacteria (*Kandleria* and *Sharpea*) also known to produce lactate as a fermentation end product.

Family/Order	Genus/Species	Strain	Culture collection #	Origin	Comments	Predicted antimicrobial biosynthetic clusters	References
Enterococcaceae	*Enterococcus faecalis*	68A		Sheep rumen/NZ			Hudson et al., 1995
Enterococcaceae	*Enterococcus gallinarum*	SKF1		Sheep rumen/NZ		Lantipeptide	Morvan and Joblin, 2000
Enterococcaceae	*Enterococcus mundtii*	C2		Cow rumen/NZ		Bacteriocin	
Enterococcaceae	*Enterococcus* sp.	KPPR-6		Cow rumen/NZ		Bacteriocin, NRPS	
Erysipelotrichaceae	*Kandleria vitulina*	MC3001		Cow rumen/NZ			Noel, 2013
Erysipelotrichaceae	*Kandleria vitulina*	WCE2011		Cow rumen/NZ			Noel, 2013
Erysipelotrichaceae	*Kandleria vitulina*	RL2	DSM 20405	Calf rumen/UK	Type strain		Bryant et al., 1958; Sharpe et al., 1973
Erysipelotrichaceae	*Kandleria vitulina*	S3b		Sheep rumen/NZ			Attwood et al., 1998
Erysipelotrichaceae	*Kandleria vitulina*	WCC7		Cow rumen/NZ			
Erysipelotrichaceae	*Kandleria vitulina*	KH4T7		Cow rumen/NZ			
Erysipelotrichaceae	*Sharpea azabuensis*	RL1	DSM 20406	Calf rumen/USA			Bryant et al., 1958
Erysipelotrichaceae	*Sharpea azabuensis*	KH1P5		Cow rumen/NZ			
Erysipelotrichaceae	*Sharpea azabuensis*	KH2P10		Cow rumen/NZ			
Lactobacillaceae	*Lactobacillus brevis*	AG48		Sheep rumen/NZ		Lantipeptide	Hudson et al., 2000
Lactobacillaceae	*Lactobacillus mucosae*	AGR63		Cow rumen/NZ			Morvan and Joblin, 2000
Lactobacillaceae	*Lactobacillus mucosae*	WCC8		Cow rumen/NZ			
Lactobacillaceae	*Lactobacillus mucosae*	KHPC15		Cow rumen/NZ			
Lactobacillaceae	*Lactobacillus mucosae*	KHPX11		Cow rumen/NZ			
Lactobacillaceae	*Lactobacillus plantarum*	AG30		Sheep rumen/NZ			Hudson et al., 2000
Lactobacillaceae	*Lactobacillus ruminis*	RF1	DSM 20403	Cow rumen/UK	Type strain	Bacteriocin	Sharpe et al., 1973
Lactobacillaceae	*Lactobacillus ruminis*	WC1T17		Cow rumen/NZ			
Lactobacillaceae	*Lactobacillus ruminis*	RF3	ATCC 27782	Cow rumen/UK		Bacteriocin	Forde et al., 2011
Lactobacillaceae	*Pediococcus acidilactici*	AGR20		Sheep rumen/NZ			Morvan and Joblin, 2000
Streptococcaceae	*Lactococcus lactis* subsp. *cremoris*	DPC6856		Cow rumen/Ireland		Bacteriocin	Cavanagh et al., 2015
Streptococcaceae	*Lactococcus lactis* subsp. *lactis*	511		Cow rumen/NZ		Lantipeptide (nisin)	Kelly et al., 2010
Streptococcaceae	*Streptococcus equinus*	B315		Sheep rumen/NZ		Lantipeptide X2	Reilly et al., 2002
Streptococcaceae	*Streptococcus equinus*	SN033		Deer rumen/NZ		Lantipeptide X3	
Streptococcaceae	*Streptococcus equinus*	AG46		Sheep rumen/NZ			Hudson et al., 2000
Streptococcaceae	*Streptococcus equinus*	2B		Sheep rumen/UK			Oxford, 1958
Streptococcaceae	*Streptococcus equinus*	JB1		Cow rumen/USA		Bacteriocin	Russell and Baldwin, 1978
Streptococcaceae	*Streptococcus equinus*	GA-1		Cow rumen/NZ		Lantipeptide X2	
Streptococcaceae	*Streptococcus equinus*	pGA-7		Cow rumen/NZ		Bacteriocin, Lantipeptide	
Streptococcaceae	*Streptococcus equinus*	pR-5		Cow rumen/NZ		Lantipeptide	
Streptococcaceae	*Streptococcus equinus*	ES1		Sheep rumen/UK		Lantipeptide	Marounck and Wallace, 1984
Streptococcaceae	*Streptococcus equinus*	C277		Sheep rumen/UK		Bacteriocin, Lantipeptide	Wallace and Brammall, 1985
Streptococcaceae	*Streptococcus equinus*	H24		Calf rumen/USA		Lantipeptide	Boyer, 1969
Streptococcaceae	*Streptococcus equinus*	Sb04		Cow rumen/Australia		Bacteriocin	Klieve et al., 1999

(Continued)

TABLE 1 | Continued

Family/Order	Genus/Species	Strain	Culture collection #	Origin	Comments	Predicted antimicrobial biosynthetic clusters	References
Streptococcaceae	*Streptococcus equinus*	Sb05		Cow rumen/Australia		Bacteriocin	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb10		Cow rumen/Australia		Bacteriocin, NRPS	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb13		Cow rumen/Australia		Lantipeptide	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb17		Cow rumen/Australia		Bacteriocin	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb18		Cow rumen/Australia			Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb20		Cow rumen/Australia		Bacteriocin	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	YE01		Goat rumen/Australia			Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	Sb09		Goat rumen/Australia		Bacteriocin	Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	SI		Sheep rumen/Australia			Klieve et al., 1999
Streptococcaceae	*Streptococcus equinus*	AR3		Sheep rumen/Australia		Bacteriocin, Lantipeptide	Klieve et al., 1989
Streptococcaceae	*Streptococcus equinus*	HC5		Cow rumen/USA		Lantipeptide	Azevedo et al., 2015
Streptococcaceae	*Streptococcus gallolyticus*	TPC2.3	LMG 15572	Goat rumen/Australia		Bacteriocin	Brooker et al., 1994; Sly et al., 1997
Streptococcaceae	*Streptococcus henryi*	A-4		Cow rumen/NZ		Lantipeptide, Thiopeptide	

All strains were sequenced as part of the Hungate1000 project (Seshadri et al., 2018) with the exceptions of L. ruminis RF3, L. lactis subsp. cremoris DPC68656 and S. equinus HC5.

body weight gain and improve feed efficiency (Frizzo et al., 2011). The meta-analysis further revealed that LAB can induce further beneficial effects if administered with whole milk and as a single strain inoculum. The use of DFM supplementation in young ruminants is expanding as farmers look to use natural alternatives to antibiotics to help improve calf health and promote growth.

In the adult ruminant, there is limited research available on the efficacy of LAB DFMs. Their use is targeted at improving the health and performance of animals (**Table 2**). With regard to health, a meta-analysis of trials evaluating the use of DFMs (predominantly *Lactobacillus*) to reduce the prevalence of *Escherichia coli* O157 fecal shedding in beef cattle has shown LAB supplementation to be efficacious (Wisener et al., 2015). Administration of *Lactococcus lactis* has been shown to be as effective as common antibiotics in the treatment of bovine mastitis (Klostermann et al., 2008). LAB DFMs have also been shown to minimize the risk of ruminal acidosis in some instances (Ghorbani et al., 2002; Lettat et al., 2012). A recent review by Rainard and Foucras (2018) appraised the use of probiotics for mastitis control. The authors concluded that based on the lack of scientific data the use of probiotics to prevent or treat mastitis is not currently recommended. However, use of teat apex probiotics deserves further research. The results from a small number of trials using only LAB supplementation treatment groups to enhance animal performance are mixed (**Table 2**). Studies where beneficial effects have been reported include an increase in milk yield, change in milk fat composition, improved feed efficiency, and increased daily weight gain but equally there have been studies where no change has been reported (see **Table 2**). Although responses to DFMs have been positive in some experiments, the basic mechanisms underlying these beneficial effects are not well defined or clearly understood.

LAB are the dominant silage inoculant in many parts of the world. LAB are used not only for their convenience and safety, but also because they are effective in controlling microbial events during silage fermentation (Muck et al., 2018). In the ensiling process, a succession of LAB ferment the available soluble sugars in cut plant material to produce organic acids, including lactic acid. As a result, the pH drops, preventing further microbial degradation of the plant material and preserving it as silage. The efficacy of adding LAB inoculants in enhancing the natural silage preservation process is well established. In addition, silage inoculants containing homofermentative LAB have not only improved silage quality and reduced fermentation losses but have also improved animal performance by increasing milk yield, daily gain and feed efficiency (Kung et al., 1993; Weinberg and Muck, 1996, 2013; Kung and Muck, 1997; Muck et al., 2018). The mechanism(s) behind the additional benefits in animal performance from feeding inoculated silage are not understood.

LAB DFMs and silage inoculants are microbial based technologies which are widely accepted and actively used in modern farming systems today. If LAB can be found to reduce ruminant CH_4 production effectively then both DFMs and inoculants provide a practical and useful mitigation option on-farm.

TABLE 2 | Animal trials which studied the effect of DFM supplementation containing LAB only on ruminant performance and health.

Target	Genus	Sector	Animal	N	Treatment/Dose/Strain	Duration of trial	Effect	References Year
Performance	*Lactobacillus plantarum* *Lactobacillus casei*	Dairy	Holstein cows	20	Treatments: (1) Control (2) 1.3×10^9 cfu/g *Lactobacillus plantarum* P-8 *Lactobacillus casei* Zhang	30 days	LAB treatment increased milk produced and certain milk functional components (IgG, lactoferrin, lysozyme, lactoperoxidase)	Xu et al., 2017
Health	*Lactobacillus rhamnosus*, *Pedioccocus acidilactici*, *Lactobacillus reuteri*	Dairy	Holstein cows	20	Treatments given intravaginally: (1) *L. rhamnosus* CECT 278, *P. acidilactici* CECT 5915, and *L. reuteri* DSM 20016, with a final cell count of 4.5×10^{10}cdu/dose and a relationship among the 3 probiotics of 12:12:1, respectively; (2) control.	3 weeks	Vaginal application of LAB maybe capable of modulating the pathogenic environment in the vaginal tract.	Genís et al., 2017
Performance	*Propionibacterium* *Lactobacillus plantarum* *Lactobacillus rhamnosus*	Dairy	Holstein cows	8	Treatments: (1) lactose (control); (2) 10^{10} cfu/d *Propionibacterium* P63; (3) 10^{10} cfu/d of both *Propionibacterium* P63 and *Lactobacillus plantarum* 115; (4) 10^{10} cfu/d of both *Propionibacterium* P63 and *Lactobacillus rhamnosus* 32	4 weeks	Some effects on CH_4 production, ruminal PH and milk FA profile but results depended on DFM strain and diet.	Philippeau et al., 2017
Performance	*Lactobacillus acidophilus,* *Lactobacillus casei* *Bifidobacterium thermophilum* *Enterococcus*	Dairy	Ewes	16	Treatments: (1) control; (2) *Lactobacillus acidophilus* ($2{\cdot}5 \times 10^7$ CFU/g), *Lactobacillus casei* ($2{\cdot}5 \times 10^7$ CFU/g), *Bifidobacterium thermophilum* ($2{\cdot}5 \times 10^7$ CFU/g), and *Enterococcus faecium* ($2{\cdot}5 \times 10^7$ CFU/g	10 weeks	Supplementing ewes with DFM products has very minor effects on milk fatty acid profiles	Payandeh et al., 2017
Health	*Lactobacillus sakei* *Pediococcus acidilactici*	Dairy	Holstein cows	100	Treatments given intravaginally: (1 and 2) *L. sakei* FUA3089, *P. acidilactici* FUA3138, and *P. acidilactici* FUA3140 with a cell count of $10^8 - 10^9$ cfu/dose; (3) control	10 weeks	LAB treatment lowered the incidence of metritis and total uterine infections.	Deng et al., 2015
Performance	*Lactobacillus acidophilus* *Propionibacterium freudenreichii*	Dairy	Holstein cows	112	Treatments: (1) control; (2) 1 g/cow per day of 1×10^9 cfu/g *Lactobacillus acidophilus* NP51 and 2×10^9 cfu/g *Propionibacterium freudenreichii* NP24	10 weeks	Supplementing cows with DFM products did not affect cow performance	Ferraretto and Shaver, 2015

(Continued)

TABLE 2 | Continued

Target	Genus	Sector	Animal	N	Treatment/Dose/Strain	Duration of trial	Effect	References Year
Performance	*Propionibacterium acidipropionici*	Beef	Heifers	20	Treatments: (1) Control; (2) *Propionibacterium acidipropionici* strain P169; (3) *P. acidipropionici* strain P5; (4) *Propionibacterium jensenii* strain P54. Inoculae of each strain (5×10^9 cfu) were administered daily.	28 days	Total and major volatile fatty acid profiles were similar among all treatments. No effects were observed on dry matter intake and total tract digestibility of nutrients. Total enteric CH_4 production (g/day) was not affected.	Vyas et al., 2014
Health	Propionibacterium *Lactobacillus plantarum Lactobacillus rhamnosus*	Sheep	Texel wethers	12	Treatments: (1) control; (2) *Propionibacterium* P63; (3) *L. plantarum* strain 115 plus P63 4) *L. rhamnosus* strain 32 plus P63. Treatment administered at a dose of 1×10^{11} cfu/wether/d.	24 days	LAB treatments may be effective in stabilizing ruminal pH and therefore preventing SARA risk, but they were not effective against lactic acidosis.	Lettat et al., 2012
Performance	*Lactobacillus acidophilus Propionibacterium freudenreichii*	Dairy	Holstein	60	Treatments: (1) control; (2) 4×10^9 cfu/head *Lactobacillus acidophilus* NP51 and *Propionibacterium freudenreichii* NP24 (3) DFM plus glycerol	10 weeks	LAB treatments improved milk and protein yield, energy corrected milk	Boyd et al., 2011
Health and performance	*Lactobacillus plantarum*	Dairy	Female goats of Damascus breed	24	Goats were assigned to one of 2 treatments (1) 10^{12} cfu/day of *L. plantarum* PCA 236 (2) control	5 weeks	LAB treatment resulted in a decrease in fecal clostridia populations and a significantly higher content of polyunsaturated fatty acids in milk fat composition	Maragkoudakis et al., 2010
Health	*Lactococcus lactis*	Dairy	Holstein Friesian cows	6	5-ml suspension (containing 10^8 cfu *L. lactis* DPC 3147) was infused into cow teat	400 h	Infusion with a live culture of a *L. lactis* lead to a rapid and considerable innate immune response.	Beecher et al., 2009
Performance	Propionibacterium	Dairy	Holstein cows	50	Treatments: (1) control; (2) Propionibacterium P169 at 6×10^{11} cfu per 25g of material	17 weeks	DFM supplementation did not increase milk production nor change milk composition but did increase feed efficiency	Weiss et al., 2008
Health	*Lactococcus lactis*	Dairy	Holstein-Friesian and New Zealand Friesians, Norwegian Reds, Normandes and Montbelliards.	Trial 1: 11; Trial 2:25	The injected suspension contained approximately 9.1 ± 0.5^{10} cfu/ml of *L. lactis* DPC3147	Trial 1: 2 weeks; Trial 2: 8 months	Of the 25 cases treated with the culture, 15 did not exhibit clinical signs of the disease following treatment. The results of these trials suggest that live culture treatment with *L. lactis* DPC3147 may be as efficacious as common antibiotic treatments in some instances.	Klostermann et al., 2008
Performance	*Lactobacillus acidophilus Propionibacteria freudenreichii*	Dairy	Holstein cows	57	Cows were randomly assigned to one of three diets. (1) 1×10^9 cfu/d *L. acidophilus* strain LA747 and 2×10^2 cfu/day *P. freudenreichii* strain PF2f. (2) 1×10^9 cfu/day *L. acidophilus* strain LA747, 2×10^9 cfu/day *P. freudenreichii* strain PF2f. (3) lactose (control)	28 days	Supplementing cows with DFM products did not affect cow performance, digestibility or rumen fermentation.	Raeth-Knight et al., 2007

(Continued)

TABLE 2 | Continued

Target	Genus	Sector	Animal	N	Treatment/Dose/Strain	Duration of trial	Effect	References Year
Performance	*Propionibacterium*	Dairy	Holstein	44	Cows were randomly assigned to one of 3 treatments (1) control (2) 6×10^{10} cfu/cow of *Propionibacterium* P169 (3) 6 × 1011 cfu/cow of P169	30 weeks	DFM supplementation enhanced ruminal digestion of forage and early lactation cows receiving supplementation produced more milk but experienced a lower, but not depressed, fat percentage.	Stein et al., 2006
Performance	*Lactobacillus acidophilus Propionibacterium freudenreichii*	Beef	Steer cattle	Trial 1: 240 Trial 2: 660	Trial 1: four treatments (1) control, (2) 1×10^9 cfu of *L. acidophilus* NP51 plus 1×10^6 cfu of *L. acidophilus* NP45 plus 1×10^9 cfu of *P. freudenreichii* NP24 per animal daily, (3) 1 × 109 cfu of *L. acidophilus* NP51 plus 1×10^9 cfu of *P. freudenreichii* NP24 per animal daily (4) 1×10^6 cfu of *L. acidophilus* NP51 plus 1×10^6 cfu *L. acidophilus* NP45 plus 1×10^9 cfu of *P. freudenreichii* NP24 per animal daily. Trial 2: three treatments (1) control (2) 5×10^6 cfu of *L. acidophilus* NP51 plus 5×10^6 cfu of *L. acidophilus* strain NP45 plus 1×10^9 cfu of *P. freudenreichii* NP24 per animal daily (3) 1×10^9 cfu of *L. acidophilus* NP51 plus 5×10^6 cfu *L. acidophilus* NP45 plus 1×10^9 cfu of *P. freudenreichii* NP24 per animal daily.	140 days	Overall, DFM supplementation did not greatly affect feedlot performance and carcass characteristics	Elam et al., 2003
Health	Propionibacterium *Enterococcus faecium*	Beef	Steer cattle	6	Treatments: (1) control, (2) *Propionibacterium* P15,(3) *Propionibacterium* P15 plus *Enterococcus faecium* EF212. Dose of 1×10^9 cfu/g	20 days	DFM supplementation did not affect blood pH and blood glucose, however, steers fed the treatment had lower concentrations of blood CO_2 than control steers, which is consistent with a reduced risk of metabolic acidosis.	Ghorbani et al., 2002
Performance	*Lactobacillus acidophilus Propionibacterium freudenreichii*	Beef	Heifers	450	Treatments: (1) control; (2) 5×10^8 cfu/head/d *L. acidophilus* BG2FO4; (3) 1×10^9 cfu/head/d *P. freudenreichii* P-63; (4) 5×10^8 cfu/head/d *L. acidophilus* BG2FO4 and 1×10^9 cfu/head/d *freudenreichii* P-63; (5) 5×10^8 cfu/head/d *L. acidophilus* BG2FO4 and 1×10^9 cfu/head/d P. *freudenreichii* P-63	126 days	Combined DFM supplementation resulted in significant improvements in daily gain and feed efficiency	Huck et al., 2000

Trials related to the use of LAB supplementation to reduce shedding of E. coli O157:H7 in beef cattle are not listed but can be found in the meta-analysis performed by Wisener et al. (2015).

Methanogens and the Rumen

Rumen methanogenic archaea are much less diverse than rumen bacteria (Henderson et al., 2015), and members of two clades of the genus *Methanobrevibacter* (referred to as *M. gottschalkii* and *M. ruminantium*) make up ∼75% of the archaeal community (Janssen and Kirs, 2008; Henderson et al., 2015). Cultivated members of both of these methanogen clades are hydrogenotrophic and use H_2 and CO_2 for CH_4 formation. Their cell walls contain pseudomurein and have similarities to those found in Gram positive bacteria which may be relevant to their sensitivity to antimicrobial agents (Varnava et al., 2017). Other significant members of the methanogen community in the rumen are methylotrophs, producing CH_4 from methyl-containing substrates, particularly methylamines and methanol. These include strains of the genus *Methanosphaera* and members of the family *Methanomassiliicoccaceae*. The former have pseudomurein-containing cell walls, while the cell envelope surrounding the *Methanomassiliicoccaceae* has not been characterized. The ability of rumen bacteria to produce the H_2 or methyl-containing substrates required for methanogenesis has been determined from culture studies, or is able to be inferred from genome sequences, but it is not yet known which bacteria are the most important contributors in the rumen.

How could LAB reduce ruminant CH_4 production? It is hypothesized that LAB could influence ruminal methanogenesis in three possible ways (**Figure 1**): (1) use of LAB or their metabolites to shift the rumen fermentation so that there is a corresponding decrease in CH_4 production, (2) use of LAB or their metabolites to directly inhibit rumen methanogens and (3) use of LAB or their metabolites to inhibit specific rumen bacteria that produce H_2 or methyl-containing compounds that are the substrates for methanogenesis.

How Have LAB Been Shown to Affect Ruminant CH_4 Production?

The idea that LAB can be used to reduce CH_4 production in ruminant livestock is not new. Reviews of CH_4 mitigation strategies consistently refer to this possibility (Hristov et al., 2013; Takahashi, 2013; Jeyanathan et al., 2014; Knapp et al., 2014; Varnava et al., 2017). However, research on the topic has been limited and convincing data from animal trials to support this concept are lacking. Jeyanathan et al. (2016) screened 45 bacteria, including strains of LAB, bifidobacteria and propionibacteria, in 24h rumen *in vitro* batch incubations for their ability to reduce methanogenesis. Three strains were selected for *in vivo* trials in sheep (n = 12), and one strain (*Lactobacillus pentosus* D31) showed a 13% reduction in CH_4 production (g CH_4/kg/DMI) over 4 weeks when dosed at 6×10^{10} cfu/animal/day. The mechanism of action was not determined in this study, but the ability of introduced bacterial strains to persist in the rumen environment was highlighted as an important factor. Subsequent work by Jeyanathan et al. (2019) using the same strains has shown no ability to reduce CH_4 emissions in dairy cows. A further two studies which examined LAB supplementation on CH_4 production have had mixed results. Mwenya et al. (2004) assessed the effect of feeding *Leuconostoc mesenteroides* subsp. *mesenteroides* to sheep (n = 4). Supplementation with this strain was found to increase CH_4 production (g CH_4/kg/DMI) *in vivo*. The authors did not offer any discussion as to how a LAB strain could increase CH_4 production *in vivo*. Astuti et al. (2018) evaluated 14 strains of *L. plantarum* in rumen *in vitro* experiments and identified strain U32 which had the lowest CH_4 production value when compared to the other LAB treatment groups. The authors hypothesized the addition of LAB may have stimulated the growth of lactic utilizing bacteria leading to increased production of propionic acid and a subsequent decrease in the hydrogen availability for methane production (Astuti et al., 2018).

Research conducted on bacteriocins and their ability to reduce ruminal CH_4 production has been minimal. The few bacteriocins and preparations from bacteriocin-producing lactic acid bacteria that have been examined have displayed promising results both *in vitro* and *in vivo*. Callaway et al. (1997) tested the effect of the *Lactococcus lactis* bacteriocin nisin on rumen fermentation *in vitro* and reported a 36% reduction in CH_4 production. However, later work has shown nisin to be susceptible to rumen proteases limiting its potential efficacy *in vivo* (Russell and Mantovani, 2002). One *in vivo* trial has, however, reported a 10% decrease in CH_4 emissions (g/kg DMI) in sheep (n = 4) fed this bacteriocin (Santoso et al., 2004). The trial was conducted for 15 days and the authors surmised that the reduction in CH_4 was due to the inhibition of growth of the methanogenic microbes. Nollet et al. (1998) examined the addition of the cell-free supernatant of *Lactobacillus plantarum* 80 (LP80) to ruminal samples *in vitro* and noted an 18% decrease in CH_4 production and a 30.6% reduction in CH_4 when the supernatant was combined with an acetogenic culture, *Peptostreptococcus productus* ATCC 35244. The effect of the LP80 supernatant in combination with *P. productus* was also studied *in vivo* using two rams and it was concluded that inhibition of methanogenesis (80% decrease; mmol/6 h) occurred during the first 3 days but the effect did not persist. Compounds (PRA1) produced by *L. plantarum* TUA1490L were tested *in vitro* and found to decrease methanogenesis by 90% (Asa et al., 2010). Further work with PRA1 confirmed its ability to maintain an antimicrobial effect even after incubation with proteases but the hypothesis that the inhibition mechanism of PRA1 may relate to the production of hydrogen peroxide has not been proven (Takahashi, 2013). Bovicin HC5, a bacteriocin produced by *Streptococcus equinus* HC5, inhibited CH_4 production by 53% *in vitro* (Lee et al., 2002), while more recently the bacteriocin pediocin produced by *Pediococcus pentosaceus* 34 was shown to reduce CH_4 production *in vitro* by 49% (Renuka et al., 2013). The possibility of using bacteriocins from rumen streptococci for CH_4 mitigation has recently been reviewed (Garsa et al., 2019). Currently, it is not clear whether the bacteriocins affect the methanogens themselves, or whether they affect the other rumen microbes that produce substrates necessary for methanogenesis. The only evidence that bacteriocins affect methanogens directly is a single article (Hammes et al., 1979) in which nisin was shown to inhibit a non-rumen methanogen, *Methanobacterium,* using an agar diffusion assay to determine the inhibitory effect. Recently, Shen et al. (2017) used *in vitro* assays and 16S rRNA gene analysis

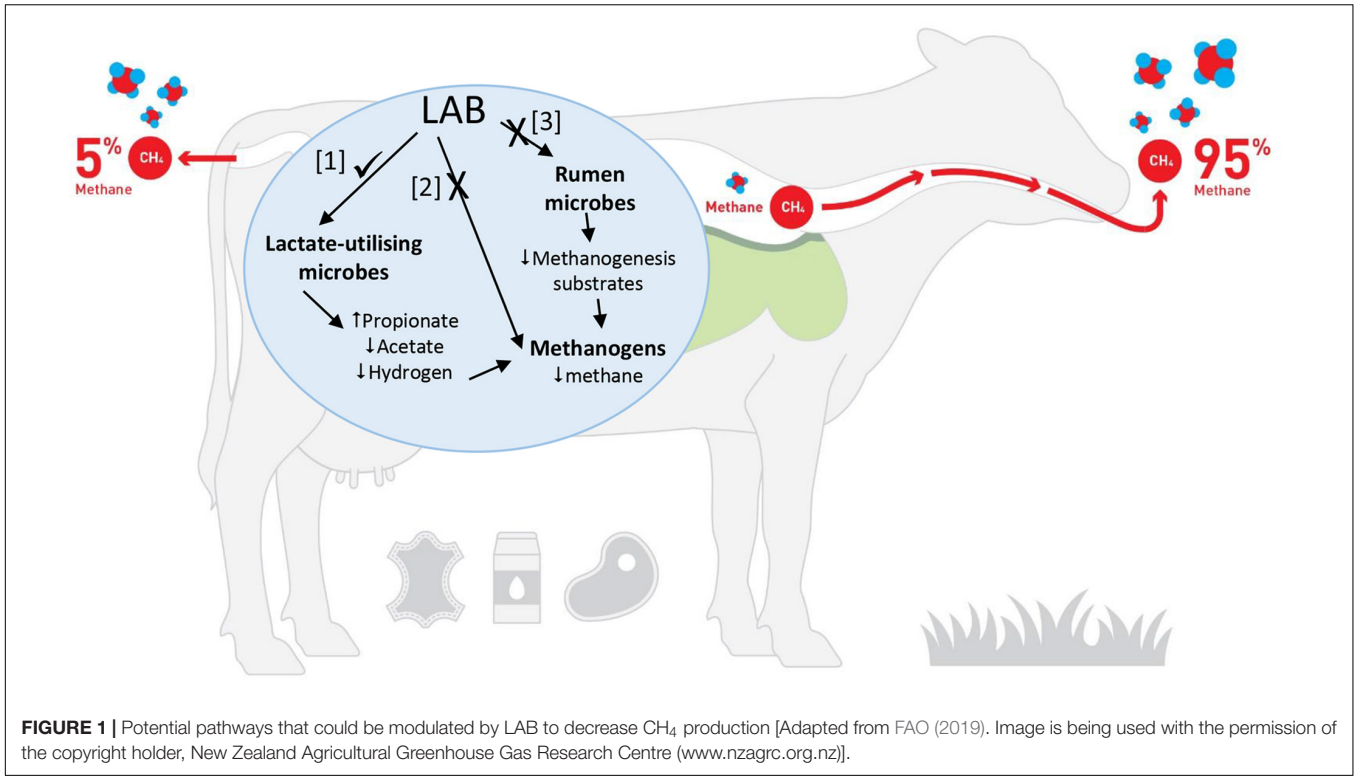


FIGURE 1 | Potential pathways that could be modulated by LAB to decrease CH_4 production [Adapted from FAO (2019). Image is being used with the permission of the copyright holder, New Zealand Agricultural Greenhouse Gas Research Centre (www.nzagrc.org.nz)].

to assess the effect nisin has on rumen microbial communities and fermentation characteristics. Results demonstrate that nisin treatments can reduce populations of total bacteria, fungi and methanogens resulting in a decrease in the ratio of acetate to propionate concentrations. A similar class of compounds (antimicrobial peptides such as human catelicidin) have also been shown to be strongly inhibitory to a range of methanogens (Bang et al., 2012, 2017). There is no standardized approach to screening methanogen cultures for their susceptibility to bacteriocins, however, the method developed to facilitate screening of small molecule inhibitors (Weimar et al., 2017) should be useful. This employs the rumen methanogen strain AbM4 (a strain of *Methanobrevibacter boviskoreani*) which grows without H_2 in the presence of ethanol and methanol (Leahy et al., 2013).

Many LAB silage inoculants possess antibacterial and/or antifungal activity and in some cases this activity is imparted into the inoculated silage (Gollop et al., 2005). The inhibitory activity has been shown to inhibit detrimental micro-organisms in silage (Flythe and Russell, 2004; Marciňáková et al., 2008; Amado et al., 2012) and has been postulated to do the same in the rumen, but the role of specific silage inoculants in CH_4 mitigation has received little attention. Thus far, research has demonstrated that LAB included in freeze-dried silage inoculants can survive in rumen fluid (Weinberg et al., 2003) and that LAB survive passage from silage into rumen fluid *in vitro* (Weinberg et al., 2004). Several studies have demonstrated that *in vitro* rumen fermentation can be altered by some LAB strains. Muck et al. (2007) made silages using a range of inoculants and showed *in vitro* that some of the inoculated silages had reduced gas production compared with the untreated silage suggesting a shift in fermentation had occurred. Cao et al. (2010a) investigated the effect of *L. plantarum* Chikuso-1 on an ensiled total mixed ration (TMR) and showed CH_4 production decreased by 8.6% and propionic acid increased by 4.8% compared with untreated TMR silage. Cao et al. (2011) found similar results with the same inoculant strain in vegetable residue silage with the inoculated silage having higher *in vitro* dry matter digestibility and lower CH_4 production (46.6% reduction). Further work with this LAB strain *in vivo* showed that the inoculated TMR silage increased digestibility and decreased ruminal CH_4 (kg DMI) emissions (24.7%) in sheep (n = 4) compared with a non-inoculated control (Cao et al., 2010b). Although more research is required in this area, the results suggest that some LAB strains are capable of altering ruminal fermentation leading to downstream effects such as reduced CH_4 production.

CONCLUSION AND FUTURE PERSPECTIVES

Literature on the use of LAB to reduce CH_4 production in ruminants is limited. In the small number of studies available, *in vitro*, LAB can reduce CH_4 production effectively. The effect is clearly strain dependent and it is not understood whether the LAB or their metabolites affect the methanogens themselves, or whether they affect the other rumen microbes that produce substrates necessary for methanogenesis. *In vivo*, the lack of robust animal trials (appropriate animal numbers, relevant treatment groups, trial period, and strain efficacy) investigating

LAB supplementation and CH_4 mitigation make it impossible at this time to make a comprehensive conclusion. Much more research is needed to understand the mechanisms behind the use of LAB as rumen modifiers. However, if appropriate LAB cultures can be identified, and proven to be effective *in vivo* then a range of delivery options that are already accepted in the global farming system such as DFMs and silage inoculants are available. This represents an alternative approach to CH_4 mitigation research and one that can be used in combination with other mitigation options such as vaccines (Wedlock et al., 2013) and CH_4 inhibitors (Dijkstra et al., 2018) which are currently under development. Ruminant production systems with low productivity lose more energy per unit of animal product than those with high productivity. In systems where farm management practices result in an increase in performance per animal (e.g., kg milk solids per cow, kg lamb slaughtered per ewe, kg beef slaughtered per cow), and combined with a reduction in stocking rates, then absolute CH_4 emissions can be reduced. LAB supplementation and use of silage inoculants can contribute to these on-farm management options that reduce agricultural GHG emissions through increases in animal productivity and improved health. LAB supplementation could offer a practical, effective and natural approach to reducing CH_4 emissions from ruminant livestock and contribute to the on-farm management practices that can be used to reduce CH_4 emissions.

AUTHOR CONTRIBUTIONS

SL, WK, and GA conceived the research. ND, PM, WK, YL, and SL performed the analysis and wrote the manuscript. RR, CS, and GA reviewed the final manuscript.

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REFERENCES

Amado, I. R., Fuciños, C., Fajardo, P., Guerra, N. P., and Pastrana, L. (2012). Evaluation of two bacteriocin-producing probiotic lactic acid bacteria as inoculants for controlling *Listeria monocytogenes* in grass and maize silages. *Anim. Feed Sci. Technol.* 175, 137–149. doi: 10.1016/j.anifeedsci.2012.05.006

Asa, R., Tanaka, A., Uehara, A., Shinzato, I., Toride, Y., Usui, N., et al. (2010). Effects of protease-resistent antimicrobial substances produced by lactic acid bacteria on rumen methanogenesis. *Asian-Australa. J. Anim. Sci.* 23, 700–707. doi: 10.5713/ajas.2010.90444

Astuti, W. D., Wiryawan, K. G., Wina, E., Widyastuti, Y., Suharti, S., and Ridwan, R. (2018). Effects of selected *Lactobacillus plantarum* as probiotic on *in vitro* ruminal fermentation and microbial population. *Pak. J. Nutr.* 17, 131–139. doi: 10.3923/pjn.2018.131.139

Attwood, G. T., Klieve, A. V., Ouwerkerk, D., and Patel, B. K. C. (1998). Ammonia-hyperproducing bacteria from New Zealand ruminants. *Appl. Environ. Microbiol.* 64, 1796–1804.

Azevedo, A. C., Bento, C. B., Ruiz, J. C., Queiroz, M. V., and Mantovani, H. C. (2015). Draft genome sequence of *Streptococcus equinus* (*Streptococcus bovis*) HC5, a lantibiotic producer from the bovine rumen. *Genome Announc.* 3:e00085-e15. doi: 10.1128/genomeA.00085-15

Bang, C., Schilhabel, A., Weidenbach, K., Kopp, A., Goldmann, T., Gutsmann, T., et al. (2012). Effects of antimicrobial peptides on methanogenic archaea. *Antimicrobial. Agents Chemother.* 56, 4123–4130. doi: 10.1128/aac.00661-12

Bang, C., Vierbuchen, T., Gutsmann, T., Heine, H., and Schmitz, R. A. (2017). Immunogenic properties of the human gut-associated archaeon *Methanomassiliicoccus luminyensis* and its susceptibility to antimicrobial peptides. *PLoS One* 12:e0185919. doi: 10.1371/journal.pone.0185919

Beecher, C., Daly, M., Berry, D. P., Klostermann, K., Flynn, J., Meaney, W., et al. (2009). Administration of a live culture of *Lactococcus lactis* DPC 3147 into the bovine mammary gland stimulates the local host immune response, particularly IL-1 and IL-8 gene expression. *J. Dairy Res.* 76, 340–348. doi: 10.1017/S0022029909004154

Bintsis, T. (2018). Lactic acid Bacteria as starter cultures: an update in their metabolism and genetics. *AIMS Microbiol.* 4, 665–684. doi: 10.3934/microbiol.2018.4.665

Boyd, J., West, J. W., and Bernard, J. K. (2011). Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. *J. Dairy Sci.* 94, 4616–4622. doi: 10.3168/jds.2010-3984

Boyer, E. W. (1969). *Amylolytic Enzymes and Selected Physiological Properties of Streptococcus bovis and Streptococcus equinus*. Ph.D. thesis, Iowa State University: Ames, IA.

Brooker, J. D., O'Donovan, L. A., Skene, I., Clarke, K., Blackall, L., and Muslera, P. (1994). *Streptococcus caprinus* sp. nov., a tannin-resistent ruminal bacterium from feral goats. Lett. Appl. Microbiol. 18, 313–318. doi: 10.1111/j.1472-765x.1994.tb00877.x

Bryant, M. P., Small, S. N., Bouma, C., and Robinson, I. (1958). Studies on the composition of the ruminal flora and fauna of young calves. *J. Dairy Sci.* 41, 1747–1767. doi: 10.3168/jds.s0022-0302(58)91160-3

Callaway, T. R., Carneiro De Melo, A. M. S., and Russell, J. B. (1997). The effect of nisin and monensin on ruminal fermentations *in vitro*. *Curr. Microbiol.* 35, 90–96. doi: 10.1007/s002849900218

Cao, Y., Cai, Y., Takahashi, T., Yoshida, N., Tohno, M., Uegaki, R., et al. (2011). Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and *in vitro* ruminal digestion of vegetable residue silage. *J. Dairy Sci.* 94, 3902–3912. doi: 10.3168/jds.2010-3623

Cao, Y., Takahashi, T., Horiguchi, K., and Yoshida, N. (2010a). Effect of adding lactic acid bacteria and molasses on fermentation quality and *in vitro* ruminal digestion of total mixed ration silage prepared with whole crop rice. *Grassl. Sci.* 56, 19–25. doi: 10.1111/j.1744-697x.2009.00168.x

Cao, Y., Takahashi, T., Horiguchi, K., Yoshida, N., and Cai, Y. (2010b). Methane emissons from sheep fed fermented or non-fermented total mixed ration containing whole-crop rice and rice bran. *Anim. Feed Sci. Tech.* 157, 72–78. doi: 10.1016/j.anifeedsci.2010.02.004

Cavanagh, D., Casey, A., Altermann, E., Cotter, P. D., Fitzgerald, G. F., and McAuliffe, O. (2015). Evaluation of *Lactococcus lactis* isolates from nondairy sources with potential dairy applications reveals extensive phenotype-genotype disparity and implications for a revised species. *Appl. Environ. Microbiol.* 81, 3961–3972. doi: 10.1128/AEM.04092-14

Cotter, D., Ross, P., and Hill, C. (2013). Bacteriocins — a viable alternative to antibiotics? *Nat. Rev. Microbiol.* 11, 95–102. doi: 10.1038/nrmicro2937

Deng, Q., Odhiambo, J. F., Farooq, U., Lam, T., Dunn, S. M., and Ametaj, B. N. (2015). Intravaginal lactic acid bacteria modulated local and systemic immune responses and lowered the incidence of uterine infections in periparturient dairy cows. *PLoS One* 10:e0124167. doi: 10.1371/journal.pone.0124167

Dijkstra, J., Bannink, A., France, J., Kebreab, E., and van Gastelen, S. (2018). Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. *J. Dairy Sci.* 101, 9041–9047. doi: 10.3168/jds.2018-14456

Eisler, M. C., Lee, M. R., Tarlton, J. F., Martin, G. B., Beddington, J., Dungait, J. A., et al. (2014). Agriculture: steps to sustainable livestock. *Nature* 507, 32–34.

Elam, N. A., Gleghorn, J. F., Rivera, J. D., Galyean, M. L., Defoor, P. J., Brashears, M. M., et al. (2003). Effects of live cultures of *Lactobacillus acidophilus* (strains NP45 and NP51) and *Propionibacterium freudenreichii* on performance, carcass, and intestinal characteristics, and *Escherichia coli* strain O157 shedding of finishing beef steers. *J. Anim. Sci.* 81, 2686–2698. doi: 10.2527/2003.81112686x

FAO, (2019). *The State of the World's Biodiversity for Food and Agriculture*, eds J. Belanger, and D. Pilling, (Rome: FAO Commission on Genetic Resources for Food and Agriculture Assessments), 572.

Ferraretto, L. F., and Shaver, R. D. (2015). Effect of direct-fed microbial supplementation on lactation performance and total-tract starch digestibility by midlactation dairy cows. *Prof. Anim. Sci.* 31, 63–67. doi: 10.15232/pas.2014-01369

Flythe, M. D., and Russell, J. B. (2004). The effect of pH and a bacteriocin (bovicin HC5) on *Clostridium sporogenes* MD1, a bacterium that has the ability to degrade amino acids in ensiled plant materials. *FEMS Microbiol. Ecol.* 47, 215–222. doi: 10.1016/S0168-6496(03)00259-9

Forde, B. M., Neville, B. A., O'Donnell, M. M., Riboulet-Bisson, E., Claesson, M. J., Coghlan, A., et al. (2011). Genome sequences and comparative genomics of two *Lactobacillus ruminis* strains from the bovine and human intestinal tracts. *Microb. Cell Fact.* 10(Suppl. 1):S13. doi: 10.1186/1475-2859-10-S1-S13

Frizzo, L. S., Soto, L. P., Zbrun, M. V., Signorini, M. L., Bertozzi, E., Sequeira, G., et al. (2011). Effect of lactic acid bacteria and lactose on growth performance and intestinal microbial balance of artificially reared calves. *Livest. Sci.* 140, 246–252. doi: 10.1016/j.livsci.2011.04.002

Garsa, A. K., Choudhury, P. K., Puniya, A. K., Dhewa, T., Malik, R. K., and Tomar, S. K. (2019). Bovicins: the bacteriocins of streptococci and their potential in methane mitigation. *Probiotics Antimicrob Proteins* doi: 10.1007/s12602-018-9502-z [Epub ahead of print].

Genís, S., Bach, À, and Arís, A. (2017). Effects of intravaginal lactic acid bacteria on bovine endometrium: implications in uterine health. *Vet. Microbiol.* 204, 174–179. doi: 10.1016/j.vetmic.2017.04.025

Ghorbani, G. R., Morgavi, D. P., Beauchemin, K. A., and Leedle, J. A. Z. (2002). Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *J. Anim. Sci.* 80, 1977–1985. doi: 10.2527/2002.8071977x

Gollop, N., Zakin, V., and Weinberg, Z. G. (2005). Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. *J. Appl. Microbiol.* 98, 662–666. doi: 10.1111/j.1365-2672.2004.02504.x

Hammes, W. P., Winter, J., and Kandler, O. (1979). The sensitivity of the pseudomurein-containing genus *Methanobacterium* to inhibitors of murein synthesis. *Arch. Microbiol.* 123, 275–279. doi: 10.1007/bf00406661

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Global Rumen Census Collaborators, et al. (2015). Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci. Rep.* 5:14567. doi: 10.1038/srep14567

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., et al. (2013). Special topics–Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. *J. Anim. Sci.* 91, 5045–5069. doi: 10.2527/jas.2013-6583

Huck, G. L., Kreikemeier, K. K., and Ducharme, G. A. (2000). Effects of feeding two microbial additives in sequence on growth performance and carcass characteristics of finishing heifers. *Kansas Agric. Exp. Station Res. Rep*. Available at: http://hdl.handle.net/2097/4655

Hudson, J. A., Cai, Y., Corner, R. J., Morvan, B., and Joblin, K. N. (2000). Identification and enumeration of oleic acid and linoleic acid hydrating bacteria in the rumen of sheep and cows. *J. Appl. Microbiol.* 88, 286–292. doi: 10.1046/j.1365-2672.2000.00968.x

Hudson, J. A., MacKenzie, C. A. M., and Joblin, K. N. (1995). Conversion of oleic acid to 10-hydroxystearic acid by two species of ruminal bacteria. *Appl. Microbiol. Biotechnol.* 44, 1–6. doi: 10.1007/s002530050511

Iverson, W. G., and Mills, N. F. (1976). Bacteriocins of *Streptococcus bovis*. *Can. J. Microbiol.* 22, 1040–1047. doi: 10.1139/m76-151

Janssen, P. H., and Kirs, M. (2008). Structure of the archaeal community of the rumen. *Appl. Environ. Microbiol.* 74, 3619–3625. doi: 10.1128/aem.02812-07

Jeyanathan, J., Martin, C., Eugène, M., Ferlay, A., Popova, M., and Morgavi, D. P. (2019). Bacterial direct-fed microbials fail to reduce methane emissions in primiparous lactating dairy cows. *J. Ani. Sci Biotech.* 10:41. doi: 10.1186/s40104-019-0342-9

Jeyanathan, J., Martin, C., and Morgavi, D. P. (2014). The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal* 8, 250–261. doi: 10.1017/S1751731113002085

Jeyanathan, J., Martin, C., and Morgavi, D. P. (2016). Screening of bacterial direct-fed microbials for their antimethanogenic potential *in vitro* and assessment of their effect on ruminal fermentation and microbial profiles in sheep. *J. Anim. Sci.* 94, 739–750. doi: 10.2527/jas2015-9682

Kamke, J., Kittelmann, S., Soni, P., Li, Y., Tavendale, M., Ganesh, S., et al. (2016). Rumen metagenome and metatranscriptome analyses of low methane yield sheep reveals a *Sharpea*-enriched microbiome characterised by lactic acid formation and utilisation. *Microbiome* 4:56.

Kelly, W. J., Ward, L. J. H., and Leahy, S. C. (2010). Chromosomal diversity in *Lactococcus lactis* and the origin of dairy starter cultures. *Genome Biol. Evol.* 2, 729–744. doi: 10.1093/gbe/evq056

Klieve, A. V., Heck, G. L., Prance, M. A., and Shu, Q. (1999). Genetic homogeneity and phage susceptibility of ruminal strains of *Streptococcus bovis* isolated in Australia. *Lett. Appl. Microbiol.* 29, 108–112. doi: 10.1046/j.1365-2672.1999.00596.x

Klieve, A. V., Hudman, J. F., and Bauchop, T. (1989). Inducible bacteriophages from ruminal bacteria. *Appl. Environ. Microbiol.* 55, 1630–1634.

Klostermann, K., Crispie, F., Flynn, J., Ross, R. P., Hill, C., and Meaney, W. (2008). Intramammary infusion of a live culture of *Lactococcus lactis* for treatment of bovine mastitis: comparison with antibiotic treatment in field trials. *J. Dairy Res.* 75, 365–373. doi: 10.1017/S0022029908003373

Knapp, J. R., Laur, G. L., Vadas, P. A., Weiss, W. P., and Tricarico, J. M. (2014). Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. *J. Dairy Sci.* 97, 3231–3261. doi: 10.3168/jds.2013-7234

Krehbiel, C. R., Rust, S. R., Zhang, G., and Gilliland, S. E. (2003). Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. *J. Anim. Sci.* 81, 120–132.

Kung, L., Chen, J. H., Creck, E. M., and Knusten, K. (1993). Effect of microbial inoculants on the nutritive value of corn silage for lactating dairy cows. *J. Dairy Sci.* 76, 3763–3770. doi: 10.3168/jds.s0022-0302(93)77719-x

Kung, L., and Muck, R. E. (1997). *Animal Response to Silage Additives. In: Silage: Field to Feedbunk, NRAES-99*. New York, NY: Northeast Regional Agricultural Engineering Service, 200–210.

Leahy, S. C., Kelly, W. J., Li, D., Li, Y., Altermann, E., Lambie, S. C., et al. (2013). The complete genome sequence of *Methanobrevibacter* sp. ABM4. *Stand. Genom. Sci.* 8, 215–227. doi: 10.4056/sigs.3977691

Lee, S. S., Hsu, J. T., Mantovani, H. C., and Russell, J. B. (2002). The effect of bovicin HC5, a bacteriocin from *Streptococcus bovis* HC5, on ruminal methane production *in vitro*. *FEMS Microbiol. Lett.* 217, 51–55. doi: 10.1016/S0378-1097(02)01044-3

Lettat, A., Nozière, P., Silberberg, M., Morgavi, D. P., Berger, C., and Martin, C. (2012). Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. *BMC Microbiol.* 12:142. doi: 10.1186/1471-2180-12-142

Mangoni, M., and Shai, Y. (2011). Short native antimicrobial peptides and engineered ultrashort lipopeptides: similarities and differences in cell specificities and modes of action. *Cell. Mol. Life Sci.* 68, 2267–2280. doi: 10.1007/s00018-011-0718-2

Mantovani, H. C., Kam, D. K., Ha, J. K., and Russell, J. B. (2001). The antibacterial activity and sensitivity of *Streptococcus bovis* strains isolated from the rumen of cattle. *FEMS Microbiol. Ecol.* 37, 223–229. doi: 10.1016/s0168-6496(01)00166-0

Maragkoudakis, P. A., Mountzouris, K. C., Rosu, C., Zoumpopoulou, G., Papadimitriou, K., Dalaka, E., et al. (2010). Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats–a preliminary study. *Int. J. Food Microbiol.* 141(Suppl. 1), S109–S116. doi: 10.1016/j.ijfoodmicro.2010.03.007

Marciňáková, M., Lauková, A., Simonová, M., Strompfová, V., Koréneková, B., and Nad, P. (2008). A new probiotic and bacteriocin-producing strain of *Enterococcus faecium* EF9296 and its use in grass ensiling. *Czech J. Anim. Sci.* 53, 335–344. doi: 10.17221/348-cjas

Marounck, M., and Wallace, R. J. (1984). Influence of culture Eh on the growth and metabolism of the rumen bacteria *Selenomonas ruminantium*, *Bacteroides amylophilus*, *Bacteroides succinogenes* and *Streptococcus bovis* in batch culture. *J. Gen. Microbiol.* 130, 223–229. doi: 10.1099/00221287-130-2-223

Morvan, B., and Joblin, K. N. (2000). Hydration of oleic acid by *Enterococcus gallinarum*, *Pediococcus acidilactici* and *Lactobacillus* sp. *Anaerobe* 5, 605–611. doi: 10.1006/anae.1999.0306

Muck, R. E., Filya, I., and Contreras-Govea, F. E. (2007). Inoculant effects on alfalfa silage: *In vitro* gas and volatile fatty acid production. *J. Dairy Sci.* 90, 5115–5125. doi: 10.3168/jds.2006-878

Muck, R. E., Nadeau, E. M. G., McAllister, T. A., Contreras-Govea, F. E., Santos, M. C., and Kung, L. (2018). Silage review: recent advances and future uses of silage additives. *J. Dairy Sci.* 101, 3980–4000. doi: 10.3168/jds.2017-13839

Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I., et al. (2004). Effects of including β1-4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. *Anim. Feed Sci. Tech.* 115, 313–326. doi: 10.1016/j.anifeedsci.2004.03.007

Noel, S. (2013). *Cultivation and Community Composition Analysis of Plant-adherent Rumen Bacteria*. Ph.D. thesis, Massey University: Palmerston North.

Nollet, L., Mbanzamihigo, L., Demeyer, D., and Verstraete, W. (1998). Effect of the addition of *Peptostreptococcus productus* ATCC 35244 on reductive acetogenesis in the ruminal ecosystem after inhibition of methanogenesis by cell-free supernatant of *Lactobacillus plantarum* 80. *Anim. Feed Sci. Tech.* 71, 49–66. doi: 10.1016/s0377-8401(97)00135-1

Opio, C., Gerber, P., Mottet, A., Falcucci, A., Tempio, G., MacLeod, M., et al. (2013). *Greenhouse Gas Emissions from Ruminant Supply Chains – A Global Life Cycle Assessment*. Rome: Food and Agriculture Organization of the United Nations (FAO).

Oxford, A. E. (1958). The nutritional requirements of rumen strains of *Streptococcus bovis* considered in relation to dextran synthesis from sucrose. *J. Gen. Microbiol.* 19, 617–623. doi: 10.1099/00221287-19-3-617

Payandeh, S., Kafilzadeh, F., Juárez, M., De La Fuente, M. A., Ghadimi, D., and Martínez Marín, A. L. (2017). Probiotic supplementation effects on milk fatty acid profile in ewes. *J. Dairy Res.* 84, 128–131. doi: 10.1017/S0022029917000115

Philippeau, C., Lettat, A., Martin, C., Silberberg, M., Morgavi, D. P., Ferlay, A., et al. (2017). Effects of bacterial direct-fed microbials on ruminal characteristics, methane emission, and milk fatty acid composition in cows fed high- or low-starch diets. *J. Dairy Sci.* 100, 2637–2650. doi: 10.3168/jds.2016-11663

Raeth-Knight, M. L., Linn, J. G., and Jung, H. G. (2007). Effect of direct-fed microbials on performance, diet digestibility, and rumen characteristics of Holstein dairy cows. *J. Dairy Sci.* 90, 1802–1809. doi: 10.3168/jds.2006-643

Rainard, P., and Foucras, G. (2018). A critical appraisal of probiotics for mastitis control. *Front. Vet. Sci.* 5:251. doi: 10.3389/fvets.2018.00251

Reilly, K., Carruthers, V. R., and Attwood, G. T. (2002). Design and use of 16S ribosomal DNA-directed primers in competitive PCRs to enumerate proteolytic bacteria in the rumen. *Microb. Ecol.* 43, 259–270. doi: 10.1007/s00248-001-1052-2

Reisinger, A., and Clark, H. (2018). How much do direct livestock emissions actually contribute to global warming? *Glob. Change Biol.* 24, 1749–1761. doi: 10.1111/gcb.13975

Renuka, Puniya, M., Sharma, A., Malik, R., Upadhyay, R. C., and Puniya, A. K. (2013). Influence of pediocin and enterocin on in-vitro methane, gas production and digestibility. *Int. J. Curr. Microbiol. Appl. Sci.* 2, 132–142.

Russell, J. B., and Baldwin, R. L. (1978). Substrate preferences in rumen bacteria: evidence of catabolite regulatory mechanisms. *Appl. Environ. Microbiol.* 36, 319–329.

Russell, J. B., and Mantovani, H. C. (2002). The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. *J. Mol. Microbiol. Biotechnol.* 4, 347–355.

Salvetti, E., Felis, G. E., Dellaglio, F., Castioni, A., Torriani, S., and Lawson, P. A. (2011). Reclassification of *Lactobacillus catenaformis* (Eggerth 1935) Moore and Holdeman 1970 and *Lactobacillus vitulinus* Sharpe et al., 1973 as *Eggerthia catenaformis* gen. nov., comb. nov. and *Kandleria vitulina* gen. nov., comb. nov., respectively. *Int. J. Syst. Evol. Microbiol.* 61, 2520–2524. doi: 10.1099/ijs.0.029231-0

Santoso, B., Mwenya, B., Sar, C., Gamo, Y., Kobayashi, T., Morikawa, R., et al. (2004). Effects of supplementing galacto-oligosaccharides, *Yucca schidigera* or nisin on ruminal methanogenesis, nitrogen and energy metabolism in sheep. *Livest. Prod. Sci.* 91, 209–217. doi: 10.1016/j.livprodsci.2004.08.004

Seshadri, R., Leahy, S. C., Attwood, G. T., Teh, K. H., Lambie, S. C., Cookson, A. L., et al. (2018). Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. *Nat. Biotechnol.* 36, 359–367. doi: 10.1038/nbt.4110

Sharpe, M. E., Latham, M. J., Garvie, E. I., Zirngibl, J., and Kandler, O. (1973). Two new species of *Lactobacillus* isolated from the bovine rumen, *Lactobacillus ruminis* sp. nov. and *Lactobacillus vitulinus* sp. nov. *J. Gen. Microbiol.* 77, 37–49. doi: 10.1099/00221287-77-1-37

Shen, J., Liu, Z., Yu, Z., and Zhu, W. (2017). Monensin and nisin affect rumen fermentation and microbiota differently *in vitro*. *Front. Microbiol.* 8:111. doi: 10.3389/fmicb.2017.01111

Signorini, M. L., Soto, L. P., Zbrun, M. V., Sequeira, G. J., Rosmini, M. R., and Frizzo, L. S. (2012). Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. *Res. Vet. Sci.* 93, 250–258. doi: 10.1016/j.rvsc.2011.05.001

Sly, L. I., Cahill, M. M., Osawa, R., and Fujisawa, T. (1997). The tannin-degrading species *Streptococcus gallolyticus* and *Streptococcus caprinus* are subjective synonyms. *Int. J. Syst. Bacteriol.* 47, 893–894. doi: 10.1099/00207713-47-3-893

Stein, D. R., Allen, D. T., Perry, E. B., Bruner, J. C., Gates, K. W., Rehberger, T. G., et al. (2006). Effects of feeding propionibacteria to dairy cows on milk yield, milk components, and reproduction. *J. Dairy Sci.* 89, 111–125. doi: 10.3168/jds.S0022-0302(06)72074-4

Stewart, C. S. (1992). "Lactic acid bacteria in the rumen," in *The Lactic Acid Bacteria*, ed. B. J. B. Wood, (Boston, MA: Springer), 1.

Stewart, C. S., Fonty, G., and Gouet, P. (1988). The establishment of rumen microbial communities. *Anim. Feed Sci. Technol.* 21, 69–97. doi: 10.1016/0377-8401(88)90093-4

Stilez, M. E., and Holzapfel, W. (1997). Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiol.* 36, 1–29. doi: 10.1016/s0168-1605(96)01233-0

Takahashi, J. (2013). "Lactic acid bacteria and mitigation of GHG emission from ruminant livestock," in *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes*, ed. M. Kongo, (London: IntechOpen).

Varnava, K. G., Ronimus, R. S., and Sarojini, V. (2017). A review on comparative mechanistic studies of antimicrobial peptides against archaea. *Biotechnol. Bioeng.* 114, 2457–2473. doi: 10.1002/bit.26387

Vieco-Saiz, N., Belguesmia, Y., Raspoet, R., Auclair, E., Gancel, F., Kempf, I., et al. (2019). Benefits and inputs from Lactic Acid Bacteria and their bacteriocins as alternatives to antibiotic growth promoters during food-animal production. *Front. Microbiol.* 10:57. doi: 10.3389/fmicb.2019.00057

Vyas, D., McGeough, E. J., McGinn, S. M., McAllister, T. A., and Beanchemin, K. A. (2014). Effect of *Propionibacterium* spp. on ruminal fermentation, nutrient digestibility, and methane emissions in beef heifers fed a high-forage diet. *J. Anim. Sci.* 92, 2192–2201. doi: 10.2527/jas2013-7492

Wallace, R. J., and Brammall, M. L. (1985). The role of different species of bacteria in the hydrolysis of protein in the rumen. *Microbiology* 131, 821–832. doi: 10.1099/00221287-131-4-821

Wedlock, D. N., Janssen, P. H., Leahy, S. C., Shu, D., and Buddle, B. M. (2013). Progress in the development of vaccines against rumen methanogens. *Animal* 7:(Suppl 2), 244–252. doi: 10.1017/S1751731113000682

Weimar, M. R., Cheung, J., Dey, D., McSweeney, C., Morrison, M., Kobayashi, Y., et al. (2017). Development of multiwell-plate methods using pure cultures of methanogens to identify new inhibitors for suppressing ruminant methane

emissions. *Appl. Environ. Microbiol.* 83:e00396-e17. doi: 10.1128/AEM.00396-17

Weinberg, Z. G., and Muck, R. E. (1996). New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiol. Rev.* 19, 53–68. doi: 10.1016/0168-6445(96)00025-3

Weinberg, Z. G., and Muck, R. E. (2013). "Potential probiotic effects of lactic acid bacteria on ruminant performance," in *Proceedings of the III International Symposium on Forage Quality and Conservation*, (Piracicaba, SP: FEALQ), 47–68.

Weinberg, Z. G., Muck, R. E., and Weimer, P. J. (2003). The survival of silage inoculant lactic acid bacteria in rumen fluid. *J. Appl. Microbiol.* 94, 1066–1071. doi: 10.1046/j.1365-2672.2003.01942.x

Weinberg, Z. G., Muck, R. E., Weimer, P. J., Chen, Y., and Gamburg, M. (2004). Lactic acid bacteria used in inoculants for silage as probiotics for ruminants. *Appl. Biochem. Biotechnol.* 118, 1–10.

Weiss, W. P., Wyatt, D. J., and McKelvey, T. R. (2008). Effect of feeding propionibacteria on milk production by early lactation dairy cows. *J. Dairy Sci.* 91, 646–652. doi: 10.3168/jds.2007-0693

Whitford, M. F., McPherson, M. A., Forster, R. J., and Teather, R. M. (2001). Identification of bacteriocin-like inhibitors from rumen *Streptococcus* spp. and isolation and characterization of bovicin 255. *Appl. Environ. Microbiol.* 67, 569–574. doi: 10.1128/aem.67.2.569-574.2001

Wisener, L. V., Sargeant, J. M., O'Conner, A. M., Faires, M. C., and Glass-Kaastra, S. K. (2015). The use of direct-fed microbials to reduce shedding of *Escherichia coli* O157 in beef cattle: a systematic review and meta-analysis. *Zoonoses Public Health* 62, 75–89. doi: 10.1111/zph.12112

Wollenberg, E., Richards, M., Smith, P., Havlik, P., Obersteiner, M., Tubiello, F. N., et al. (2016). Reducing emissions from agriculture to meet the 2 °C target. *Glob. Chang. Biol.* 22, 3859–3864. doi: 10.1111/gcb.13340

Xu, H., Huang, W., Hou, Q., Kwok, L., Sun, A., Ma, H., et al. (2017). The effects of probiotics administration on the milk production, milk components and fecal bacteria microbiota of dairy cows. *Sci. Bull.* 62, 767–774. doi: 10.1016/j.scib.2017.04.019

Conflict of Interest: WK was employed by the company Donvis Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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