

The genetic diversity of lactic acid producing bacteria in the equine gastrointestinal tract

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Abstract

Seventy-two lactic acid producing bacterial isolates (excluding streptococci) were cultured from the gastrointestinal tract of six horses. Two of the horses were orally dosed with ractilose to induce lactic acidosis and laminitis while the remaining four were maintained on a roughage diet. Near complete 16S rDNA was amplified by PCR from the genomic DNA of each isolate. Following RFLP analysis with the restriction enzymes *Mbo*I, *Hha*I and *Hin*fI, the PCR products from the 18 isolates that produced L- and/or D-lactate were subsequently cloned and sequenced. DNA sequence analysis indicated that the majority of the isolates were closely related to species within the genus *Lactobacillus*, including *Lactobacillus salivarius*, *Lactobacillus mucosae* and *Lactobacillus delbrueckii*. Four isolates were closely related to *Mitsuokella jalaludinii*. Lactic acid producing bacteria (LAB) from the equine gastrointestinal tract was dominated by representatives from the genus *Lactobacillus*, but also included D-lactate-producing bacteria closely related to *M. jalaludinii*. Identification and characterization of LAB from the equine gastrointestinal tract should contribute to our understanding and management of fermentative acidosis, ulceration of the stomach and laminitis.

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1. Introduction

In horses fed grass or hay, fermentation of structural carbohydrates by the action of microbial enzymes in the cecum and the colon produces volatile fatty acids, which are readily absorbed and utilized as an energy source. In contrast, feeding high grain, starch rich diets, which is

particularly a problem in intensively managed racehorses, disrupts the microbial ecosystem of the hindgut. Starch is not well digested in the small intestine of the horse and variable proportions of ingested starch that escapes enzymatic digestion in the small intestine, enters the hindgut and is rapidly fermented. This increases VFA and lactate production and lowers the pH. An acidic environment favors the rapid proliferation of lactic acid producing bacteria (LAB), resulting in increased lactic acid production and a further decline in the pH.

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Lactic acidosis is often accompanied by laminitis. The key lesion of laminitis is failure of the attachment between the inner hoof wall and the distal phalanx of the foot at the lamellar dermal-epidermal junction [1]. The effect of carbohydrate overload on subsequent development of lactic acidosis and laminitis has been demonstrated [2,3]. The administration of carbohydrate (cornstarch–wood flour gruel, 85% corn starch and 15% wood cellulose flour) via a stomach tube at a rate of 17.6 g kg^{-1} body weight elevated plasma L-lactate levels in horses and induced laminitis and circulatory collapse [2]. Changes in D-lactate were not quantified, although the authors did not rule out the possibility that elevated plasma D-lactate levels may have occurred in a fashion similar to that of L-lactate and emphasized the need to measure D-lactate in future studies. More recently, it was clearly demonstrated that the administration of ground wheat at rates of 12 and 15 g kg^{-1} live weight resulted in the accumulation of L- and D-lactic acid in the cecum and colon of horses and elevated blood D-lactate [3]. Horses that had high levels of lactate in their digestive tract developed signs of lameness within 24 h of dosing with the ground wheat grain and those that developed laminitis had high levels of D-lactate in the blood. Pollitt and co-workers (personal communication) induced laminitis by alimentary overload with oligofructose (OF) and showed that after OF dosing blood D-lactate values peaked at 24 h then declined and disappeared by 40 h. This observation indicated the rapid proliferation of D-lactate producing organisms in the hindgut.

Two species of streptococcal LAB, *Streptococcus bovis* and *Streptococcus equinus*, appear to be the predominant LAB in the hindgut of horses [4]. Both are homofermentative, producing only L-lactate. Although the production of D-lactate by hindgut microbiota following carbohydrate overload has been directly observed [3] and indirectly observed following incubation of fresh equine faecal samples with glucose at 39°C for 20 h [5], bacteria that produce this D-lactate peak in the equine gastrointestinal tract have not yet been identified. Recently it was reported that *S. bovis* and *Lactobacillus salivarius* formed 3% of 272 randomly generated 16S rDNA clones obtained from the hindgut of freshly slaughtered grass-fed horses [6]. More recently, an in vitro carbohydrate overload model simulating the equine cecum was used to identify *S. bovis*, *L. mucosae*, *Lactobacillus reuteri*, *L. salivarius*, *Lactobacillus delbrueckii*, and *L. fermentum* as the key amino acid decarboxylating bacteria associated with carbohydrate overload in the cecum of horses [7]. In this paper we report on the identification, characterization and genetic diversity of the main L- and D-LAB of the equine gastrointestinal tract that are distinct from *S. bovis* and *S. equinus*, and discuss their potential contribution to lactic acidosis and laminitis.

2. Materials and methods

2.1. Animals and experimental diets

Six horses were used as the source of gastrointestinal tract contents in this study. Two mature Standardbred castrate male horses with normal feet were dosed with 12.5 g kg^{-1} live weight oligofructose (Raftilose P95®)¹ by nasogastric intubation and developed laminitis prior to slaughter at 48 h. These two horses were part of a group of eight horses used in an experiment aimed at developing a horse, hind gut acidosis and laminitis induction model using oligofructose (OF). Blood samples were collected from the two horses dosed with OF and from two sham-infected horses at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, and 48 h after dosing for the analysis of D-lactate. Four mature Australian stock horses (2 castrate males and 2 females) were fed medium quality grass hay, two of which were deprived of feed and water for 6 h prior to slaughter while the other two had access to feed and water ad libitum. Horses weighed 378 ± 5.3 (mean \pm SE) kg and were aged between 6 and 9 years. The gastrointestinal tracts of all horses were removed at post-mortem and the contents of the stomach, cecum, ventral and dorsal colons and rectum were emptied into plastic containers (50 l) and thoroughly mixed. Approximately 200 g of samples were then obtained in 50 ml plastic beakers and immediately placed in pre-warmed containers, flushed with CO_2 and transported to the laboratory.

2.2. Isolation and characterization of LAB bacteria

Upon arrival at the laboratory, the equine gastrointestinal content specimens were flushed with CO_2 and 5 g samples were homogenized in 45 ml anaerobic dilution solution (ADS) [8] using a stomacher (Stomacher 400 Circulator, Seward Ltd., Thetford, UK) for two cycles of 30 s each at 230 rpm. The homogenized contents were strained through four-layers of sterilized cheesecloth and then serially diluted ten-fold in ADS to a final dilution of 10^{-9} [8]. Four dilutions (10^{-6} , 10^{-7} , 10^{-8} and 10^{-9}) were used to inoculate modified MRS agar in roll-tubes in triplicate for each dilution. The tubes were incubated at 39°C for 48 h. The MRS medium (Oxoid, Basingstoke, UK) [9] was modified by adding reducing solution (1 ml containing 0.026 g cysteine-HCl and 0.026 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ per 100 ml of media) and adjusting the pH to 5.5 [10]. Colonies were picked from the roll tubes after 48 h growth and transferred into BM10 broth [8], containing glucose (0.3% w/v) and incubated anaerobically at 39°C for 48 h. After 24 h incubation a drop of the culture was examined microscopically to check the purity of the cultures, and gram stained. The gram morphology was recorded with mixed populations re-cultured in roll tubes and BM10 broth until pure

cultures were obtained. At this stage, any isolates with morphology consistent with gram positive cocci were excluded from further analysis.

A total of 72 isolates were purified and analyzed for fermentation end-products before further analysis. Fermentation end-products were obtained after 20 h of anaerobic incubation at 39 °C in BM10 broth supplemented with glucose (0.3% w/v). The pH was measured and the culture was acidified to pH 2 to 3 with sulphuric acid for further analysis of volatile fatty acids (VFA) and lactate. VFA concentrations were measured using flame ionization detection in a gas chromatograph (Hewlett–Packard) fitted with a Chromosorb 'W', acid washed and 60–80 mesh column coated with two liquid phases, a: *o*-phosphoric acid (1.5% v/v) and b: polypropylene glycol sebacate (17.5% v/v). The carrier gas was nitrogen and the temperatures of the column oven, detector and the injector were 135, 180 and 210 °C, respectively. L-lactate and D-lactate were analyzed with an auto-analyzer (Cobas Mira Autoanalyzer, Roche Diagnostics Inc., Frenchs Forest, Australia) using an enzymatic procedure (Stat-Pack™ Rapid Lactate Test, Cat. No. 1112 821, Behring Diagnostics Inc., Somerville, USA).

2.3. DNA extraction

Total DNA was extracted from overnight cultures (5 ml) of the selected LAB following modifications of a previously described protocol [11]. In summary, cells were harvested by centrifugation and resuspended in 467 µl of cell lysis buffer (10 mM Tris, 1 mM EDTA, pH 8.0). In 1.5 ml microfuge tubes, 30 µl of 10% (w/v) sodium dodecyl sulphate (SDS) and 3 µl of 20 mg ml⁻¹ proteinase K was added and the tubes incubated for 1 h at 37 °C. Next, nucleic acids were extracted twice by the addition of an equal volume of phenol/chloroform/isomylalcohol (25:24:1). DNA was precipitated by the addition of 0.1 volumes of 3 M sodium acetate (pH 5.2) and 0.6 volumes of cold isopropanol. After 1 h incubation at 4 °C the DNA was collected by centrifugation at 13,000 rpm for 15 min. The DNA was washed twice with 250 µl of cold 70% ethanol and the dried DNA pellet was resuspended in 100 µl of sterile Milli-Q water. The yield and quality of extracted DNA was determined by agarose gel electrophoresis.

2.4. PCR amplification and cloning of 16S rDNA

The primers 27f (5'-AGAGTTTGATCCTGGCT-CAG-3') and 1492r (5'-TACGGYTACCTTGTTACG-ACTT-3') were used to amplify the 16S rDNA [12]. PCRs were performed in a reaction volume of 20 µl containing PCR buffer (20 mM Tris–HCl, pH 8.4; 50 mM KCl), 1.5 mM MgCl₂, 0.5 mM dNTPs (Promega, Madison, USA), 0.5 µM of each primer, 1 unit of Taq

DNA Polymerase (Invitrogen, Carlsbad, USA), and 1.0 µl of genomic DNA prepared as described above. PCR amplification was performed in a PCR Express thermal cycler (Hybaid, Ashford, UK) under the following conditions; an initial cycle of 94 °C for 5 min, 60 °C for 1 min and 72 °C for 1.5 min, then 30 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1.5 min, and a final cycle of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 8 min. The PCR products were analysed by electrophoresis in a 1% agarose gel.

To determine the genetic diversity of bacterial isolates, PCR products were digested separately with the restriction enzymes *Hinf*I, *Hha*I and *Mbo*I (New England Biolabs Inc., Beverly, USA). In a reaction volume of 10 µl, PCR products were digested with *Hinf*I, *Hha*I or *Mbo*I (5, 20 and 0.5 U, respectively) for 16–18 h at 37 °C according to the manufacturer's instructions. DNA fragments were electrophoresed in 1% agarose gels. Isolates were then selected for subsequent cloning based on restriction fragment length profiles, and on morphological and fermentation characteristics.

Before cloning, PCR products of selected isolates were purified using a NucleoSpin Extract Kit (Machery-Nagel, Düren, Germany) according to the manufacturer's instructions. Purified PCR products were then cloned into pGEM-T Easy vector (Promega, Madison, USA) according to the manufacturer's instructions. Ligations were performed at room temperature for 3 h followed by transformation of *Escherichia coli* strain JM109 with the respective ligation mix [13]. Transformants were screened on LB agar plates supplemented with 50 µg ml⁻¹ ampicillin, to which 25 µl of 50 mg ml⁻¹ X-Gal (5-bromo-4-chloro-3-indoyl-β-D-galactoside) and 40 µl of 100 mM IPTG (isopropyl-β-D-thiogalactopyranoside) had been added. Plasmid DNA was isolated from selected transformants using a Mini Plasmid Prep Kit (MoBio Laboratories Inc., Solana Beach, USA) according to the manufacturer's instructions. To identify clones with inserts of the appropriate size, plasmid DNA was digested separately with the restriction enzymes *Not*I and *Eco*RI (10 and 20 U, respectively).

2.5. DNA sequence analysis

Plasmid DNA clones of near complete 16S rDNA were sequenced using the primers SP6 (5'-GCCA-GGCTATTTAGGTGACACTATAG-3'), T7 (5'-GG-GTAATACGACTCACTATAGGG-3'), 530f (5'-GT-GCCAGCMGCCGCGG-3'), 907r (5'-CCGTCAAT-TCMTTTRAGTTT-3') or 1100r (5'-GGGTTGCGCT-CGTTG-3') [12], with the ABI Prism BigDye Terminator kit, Version 3.0 or 3.1, according to the manufacturer's instructions (Applied Biosystems, Foster City, USA). Gel separation of DNA sequencing products was done at the Australian Genome Research Facility (Brisbane, Australia).

Initially, DNA sequence data sets were edited using BIOEDIT [14] and characterized by BLAST analysis for the most closely related sequences [15]. All the DNA sequence data sets from this study together with a selection of the most closely related sequences from the public databases were aligned using CLUSTAL W version 1.8 [16]. Phylogenetic and molecular analyses were then conducted on the aligned sequences using MEGA version 2.1 [17]. A neighbor-joining phylogenetic tree was constructed using the Tamura-Nei model with bootstrap analysis performed by resampling the data 1000 times. Accession numbers for 16S rDNA sequence from this study are AY391825, AY391826, AY389802–AY389804, and AY445123–AY445135.

3. Results

3.1. Bacterial isolates and fermentation end-products

Seventy-two isolates were selected on the basis of their growth on MRS agar at high dilutions (10^{-7} and 10^{-8}), and their morphology and gram stain reaction. The isolates were purified and tested for their fermentation end-products. Twenty-five of the isolates produced L-lactate, D-lactate, or both L- and D-lactate. The origin and amount of L- and D-lactate produced in vitro by each of these isolates and the recognized bacterial species to which they are most closely related are presented in Table 1. Isolates related to *Mitsuokella jalaludinii* produced the highest level of D-lactate (23.13 ± 2.0 mM) and small amounts of L-lactate (1.25 ± 0.63 mM). The

three isolates related to *L. salivarius* produced high levels of the L-isomer of lactate (21.67 ± 1.86 mM) and less of D-lactate (2.63 ± 0.41 mM). All other isolates produced small amounts of L- and/or D-lactate. Isolates that produced small amounts of lactate produced variable amounts of VFA (data not shown).

3.2. Blood D-lactate level

Horses dosed with 12.5 g kg^{-1} live weight oligofructose (OF) developed laminitis and had elevated blood D-lactate levels. Blood D-lactate values peaked at 20–24 h (2.815 mM/l) then declined and disappeared by 36–40 h (Fig. 2). Although not part of this study, horses dosed with OF at 7.5 and 10 g kg^{-1} live weight also had elevated levels of blood D-lactate peaking at 1.632 and 2.93 mM/l , respectively (Pollitt, personal communication). The detection of D-lactate in blood indicates rapid proliferation of D-lactate producing bacteria in the hind gut of the horse following dosing with OF.

3.3. 16S rDNA sequence and phylogenetic analyses

16S rDNA was amplified from each of the 25 isolates of LAB and subjected to RFLP analysis prior to sequencing. Using the restriction enzymes *HinfI*, *HhaI* and *MboI*, 18 distinct 16S rDNA RFLP patterns were identified and subjected to near complete 16S rDNA sequence analysis. Thirteen of the isolates were closely related to species from the genus *Lactobacillus* and clustered into three groups (Fig. 1). Group 1 included four isolates, one of stomach origin (RA2053) and three

Table 1

16S rDNA identification of 18 lactic acid producing bacteria isolated from the different sites of the gastrointestinal tract and their fermentation end-products

Isolate	Origin	Closest relatives ^a	Gram reaction	L-Lactate (mM)	D-Lactate (mM)	Dietary regimen
RA2053	Stomach	<i>Lactobacillus mucosae</i> 95%	+	1.0	2.2	Roughage fed ad libitum
RA2062	Stomach	<i>Lactobacillus delbrueckii</i> 98%	+	ND ^b	4.3	Roughage fed ad libitum
RA2066	Stomach	<i>L. delbrueckii</i> 98%	+	ND	1.9	Roughage fed fasted overnight
RA2070	Cecum	<i>Lactobacillus mucosae</i> >99%	+	2.0	2.7	Laminitis induced
RA2071	Cecum	<i>Lactobacillus mucosae</i> >99%	+	ND	0.5	Laminitis induced
RA2074	Cecum	<i>Mitsuokella jalaludinii</i> 98%	–	1.0	23.7	Laminitis induced
RA2083	Cecum	<i>M. jalaludinii</i> 97%	–	1.0	25.5	Laminitis induced
RA2087	Cecum	<i>Lactobacillus mucosae</i> >99%	–	1.0	1.1	Laminitis induced
RA2105	Colon	<i>Lactobacillus salivarius</i> >99%	+	24.0	2.0	Laminitis induced
RA2107	Rectum	<i>M. jalaludinii</i> 98%	–	ND	17.3	Laminitis induced
RA2108	Rectum	<i>M. jalaludinii</i> 98%	–	3.0	26.0	Laminitis induced
RA2113	Rectum	<i>L. delbrueckii</i> 96%	+	2.0	3.7	Laminitis induced
RA2114	Rectum	<i>Veillonella atypica</i> 93%	–	3.0	5.3	Laminitis induced
RA2115	Rectum	<i>L. salivarius</i> >99%	+	18.0	2.5	Laminitis induced
RA2116	Rectum	<i>L. salivarius</i> >99%	+	23.0	3.4	Laminitis induced
RA2120	Rectum	<i>L. delbrueckii</i> 98%	+	ND	3.9	Laminitis induced
T057	Stomach	<i>L. salivarius</i> 94%	+	ND	4.9	Roughage fed ad libitum
T059	Stomach	<i>L. salivarius</i> 94%	+	ND	4.6	Roughage fed fasted overnight

^a The closest relatives indicated above are completely characterized bacterial species, even though in some cases the closest relatives was either an uncultured or incompletely described bacterium.

^b ND: not detectable.

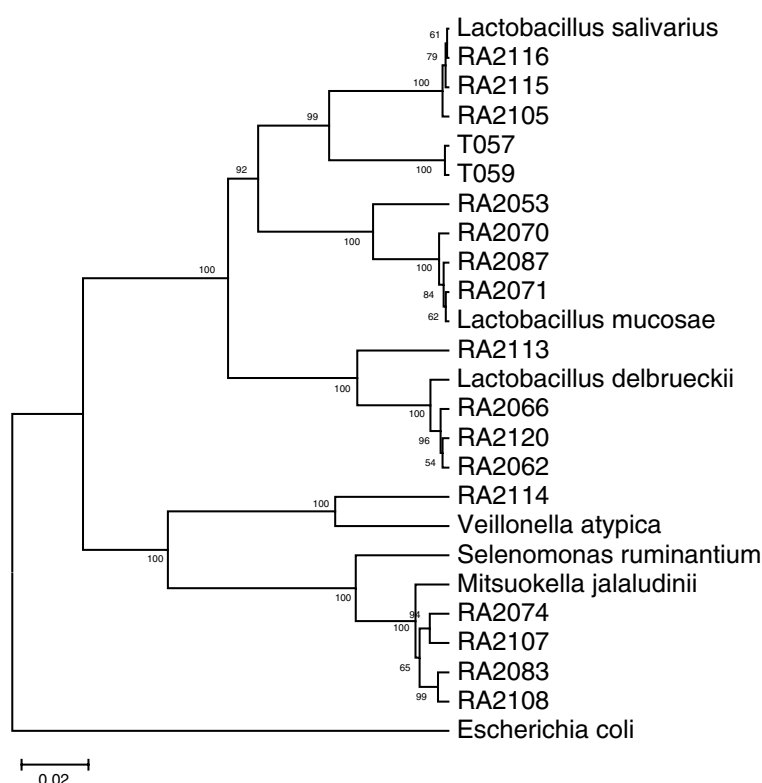


Fig. 1. Phylogenetic relationship of LAB isolates from the equine gastrointestinal tract, based on near complete 16S rDNA sequence data.

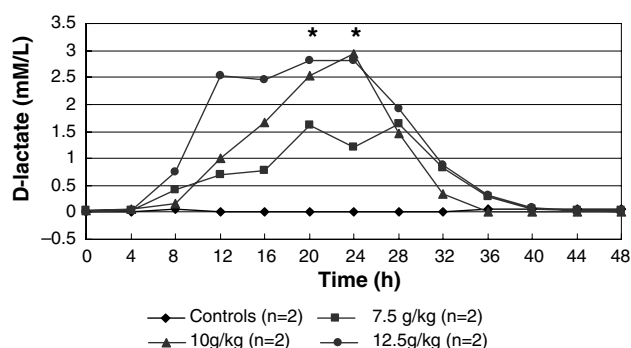


Fig. 2. Blood level of D-lactate (mmol/l) in horses dosed with the oligofructose (Raftilose P95®) at different rates. * $P < 0.05$, Horses dosed with more than 10 g kg^{-1} BWt had higher blood D-lactate levels than horses dosed with 7.5 g kg^{-1} BWt at 20 and 24 h.

from the cecum (RA2070, RA2071, and RA2087). This group shared from 95 to greater than 99% identity with *L. mucosae*. Group 2 included four isolates, two of stomach origin (RA2062 and RA2066) and two from the rectum (RA2113 and RA2120), and clustered with *L. delbrueckii* at between 96% and 98% identity. Group 3 had five isolates, with three closely related to *L. salivarius* and sharing greater than 99% identity. One of these isolates (RA2105) originated from the colon, and two were of rectal origin (RA2115 and RA2116). The two remaining isolates from group 3 (T057 and T059) were of stomach origin, but shared only 94% identity with

L. salivarius. Four of the remaining five isolates were related to *M. jalaludinii* with 97–98% identity. Two of these isolates (RA2074 and RA2083) were of cecal origin and two originated from the rectum (RA2107 and RA2108). The remaining isolate was of rectal origin (RA2114), and clustered with *Veillonella atypica* but with only 93% identity.

4. Discussion

The main aim of this study was to identify the predominant L- and D-lactic acid-producing bacteria (LAB) of the horse gastrointestinal tract other than *S. bovis* and *S. equinus*. Our ability to culture, characterize and identify the predominant LAB other than those previously reported will enhance our understanding of fermentative acidosis in horses and may lead to a better understanding of the complex microbial events that precede the development of carbohydrate-induced laminitis.

The majority of LAB identified by 16S rDNA sequencing belonged to several species in the *Lactobacillus* genus. The isolation of different species of lactobacilli from different sections of the gastrointestinal tract of laminitic horses suggests that these bacteria have the ability to adapt to a range of in vivo environments and changes in pH. *L. salivarius* and *L. mucosae* were

the predominant lactobacilli identified. The culturing of *L. salivarius* from the colon and rectal contents as well as the stomach in this study indicates the potential of this bacterium to exist in extremes of pH and suggests that it may contribute to lactic acid accumulation both in the stomach and the hindgut. *L. salivarius* has been previously shown to adhere to the lining of the stomach of the horse [18]. Recent work has shown that *L. salivarius* and *L. mucosae* derived from the equine hindgut are capable of decarboxylating amino acids, producing potentially vasoactive amines under conditions where readily fermentable carbohydrates become available for microbial fermentation [7]. These products are hypothesized to contribute to the pathophysiology of laminitis by causing vasoconstriction of digital blood vessels of the hoof, resulting in ischemia. This theory is in contrast to a previous study that showed among horses developing alimentary carbohydrate laminitis, in a temperature controlled environment, only those with a vasodilated foot circulation developed laminitis [19]. Hoof lamellar tissues affected by laminitis show upregulated expression and concomitant activation of matrix metalloproteinases (Pollitt, personal communication). These enzymes are capable of cleaving the structural proteins responsible for maintaining attachment at the hoof lamellar dermo-epidermal junction. Activation of lamellar matrix metalloproteinases may be the result of hematogenous delivery to the feet of microbial factors generated by the fermentation of excess carbohydrate in the hindgut. Support for a direct bacterial pathogenesis for laminitis comes from in vitro studies [20], where lamellar dermo-epidermal separation and matrix metalloproteinase activation resulted when lamellar explants were co-cultured with hindgut bacterial broth cultures, most notably those of *S. bovis*.

In this study another group of lactobacilli closely related to *L. delbrueckii* were also found both in the stomach and rectal contents. These bacteria closely resemble *Lactobacillus* spp. commonly found in fermented food products including yoghurt [21], artisanal Sicilian cheese [22], and malt whisky [23], and have been found in the gastrointestinal tract of many animal species including pigs [24], chickens [25] and horses [7]. Interestingly, *L. delbrueckii* was reported to be the main causative agent in the development of human gastrointestinal D-lactic acidosis [26].

We obtained a single isolate, RA2114, that showed relatively low DNA sequence similarity with the previously published *V. atypica* sequence (approximately 94%), and so has the potential of being a new species of the genus *Veillonella*. This bacterium is more closely related to *Selenomonas* and *Mitsuokella* than other LAB. A recent report has indicated that *V. atypica* is a lactate utilizer and produces propionic and acetic acids as end-products [27]. However, we have demonstrated that isolate RA2114 actually produces both L- and

D-lactate and may contribute to lactic acidosis, although the level of lactic acid production by this strain was very low when compared with the other isolates identified in the study.

Of particular interest are the D-lactate producers identified in the study that shared between 97% and 98% sequence identity with *M. jalaludinii*. These isolates have not been previously identified in the gastrointestinal tract of horses. This level of sequence identity is on the borderline for assigning these isolates within the *M. jalaludinii* species, however further genetic and phenotypic characterization, such as determining the level of DNA-DNA relative reassociation with other recognized species of *Mitsuokella*, will be required. *M. jalaludinii* was first isolated from the gastrointestinal tract of cattle in Malaysia and was named after S. Jalaludin, an animal nutritionist at Putra University in Malaysia [28]. The organism was demonstrated to produce phytase and lactate. Unfortunately, the type of lactate produced was not determined. In the present study, the isolates sharing 97–98% 16S rDNA sequence identity with *M. jalaludinii* were shown to ferment glucose and produce the D-isomer of lactate at quantities similar to the high amounts of L-lactate previously reported for *S. bovis* [4]. Furthermore, these isolates were obtained from very high dilutions (10^{-7} – 10^{-8}) of gastrointestinal tract contents of horses that were induced with laminitis and had evidence of an elevated blood D-lactate concentration. Since the animal does not express the enzyme D-lactate dehydrogenase required for the conversion of pyruvate to D-lactate or lactate racemase that catalyses the conversion of L-lactate to D-lactate, this could only come from gut microbes that have such capacity. The relative in vivo population sizes of the isolated bacterial groups and their response to increased available carbohydrate, and hence significance to lactic acidosis, is unknown at this time.

Unlike *S. bovis* and *S. equinus*, *Mitsuokella* spp. are Gram negative bacteria and therefore they are not sensitive to antibiotic feed additives such as virginiamycin that are commercially available to prevent acidosis and laminitis in horses [4]. This suggests that there will be situations where the use of virginiamycin may not provide full protection against acidosis in the horse. It will then become important to identify conditions that favor the proliferation and establishment of *M. jalaludinii* in the gastrointestinal tract of the horse to find alternative methods of control. These findings have contributed to a better understanding of the microbial ecosystem of the equine gastrointestinal tract and enhance our ability to develop strategies to monitor and treat diseases associated with lactic acid accumulation. In particular, molecular characterization of LAB will enable DNA-based assays to be developed to monitor microbial population changes involved in the onset of lactic acidosis and laminitis.

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