Factors Affecting Lactate and Malate Utilization by *Selenomonas ruminantium*

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Lactate utilization by *Selenomonas ruminantium* is stimulated in the presence of malate. Because little information is available describing lactate-plus-malate utilization by this organism, the objective of this study was to evaluate factors affecting utilization of these two organic acids by two strains of *S. ruminantium*. When *S. ruminantium* HD4 and H18 were grown in batch culture on DL-lactate and DL-malate, both strains utilized both organic acids for the initial 20 to 24 h of incubation and acetate, propionate, and succinate accumulated. However, when malate and succinate concentrations reached 7 mM, malate utilization ceased, and with strain H18, there was a complete cessation of DL-malate utilization. Malate utilization by both strains was also inhibited in the presence of glucose. *S. ruminantium* HD4 was unable to grow on 6 mM DL-lactate at extracellular pH 5.5 in continuous culture (dilution rate, 0.05 h⁻¹) and washed out of the culture vessel. Addition of 8 mM DL-malate to the medium prevented washout on 6 mM DL-lactate at pH 5.5 and resulted in succinate accumulation. Addition of malate also increased bacterial protein, acetate, and propionate concentrations in continuous culture. These results suggest that 8 mM DL-malate enhances the ability of strain HD4 to grow on 6 mM DL-lactate at extracellular pH 5.5.

*Selenomonas ruminantium* is a common gram-negative anaerobic ruminal bacterium that can account for up to 51% of the total viable bacterial count in the rumen (4). Previous research showed that *S. ruminantium* HD4 requires l-aspartate, CO₂, p-aminobenzoic acid, and biotin for growth in a lactate-salts medium (13, 14). Strain H18 requires aspartate for growth on lactate (30). However, the required aspartate can be replaced by malate or fumarate in both strains (14, 30). Neither strain can grow on aspartate, fumarate, or malate in the absence of lactate (14, 21), but both strains can grow on all three organic acids in the presence of extracellular H₂ (16).

Recent research demonstrated that l-lactate uptake by *S. ruminantium* HD4 was stimulated by 10 mM l-malate (fourfold), fumarate (fourfold), and l-malate (10-fold) (21). Different concentrations (0.03 to 10 mM) of l-malate stimulated l-lactate uptake in a dose response fashion, with 10 mM l-malate giving the greatest response (22). l-Lactate uptake in the absence of l-malate was low regardless of the Na⁺ concentration, but Na⁺ concentrations between 25 and 100 mM stimulated l-lactate uptake in the presence of 10 mM l-malate (23).

Based on the ability of malate to stimulate lactate utilization by *S. ruminantium*, experiments were conducted to evaluate the effects of DL-malate on the mixed ruminal microorganism fermentation of cracked corn and soluble starch (5, 17). Malate treatment increased the final pH and propionate concentrations and decreased lactate concentrations in these fermentations (5, 17). Even though malate stimulates lactate utilization by *S. ruminantium* and results with mixed cultures show promise, little information is available regarding factors that affect lactate-plus-malate utilization by this bacterium. Therefore, the objective of this study was to evaluate the utilization of lactate plus malate over time by *S. ruminantium* HD4 and H18 in batch culture. In addition, the effects of organic acid concentration, extracellular pH, and dilution rate on lactate-plus-malate utilization by strain HD4 were examined in continuous culture.

**MATERIALS AND METHODS**

**Organisms and growth medium.** *S. ruminantium* HD4 and H18 were used in this study. Strain HD4 was obtained from J. B. Russell, Cornell University, and strain H18 was obtained from H. J. Strobel, University of Kentucky. Each liter of basal medium (pH 6.7) contained 292 mg of K₂HPO₄, 240 mg of KH₂PO₄, 480 mg of (NH₄)₂SO₄, 480 mg of NaCl, 100 mg of MgSO₄·7H₂O, 64 mg of CaCl₂, 2H₂O, 4,000 mg of Na₂CO₃, 600 mg of cysteine hydrochloride, 1,000 mg of Trypticase (Becton Dickinson and Co., Cockeysville, Md.), 1 mg of resazurin, 500 mg of yeast extract (Difco Laboratories, Detroit, Mich.), 28.3 mM of acetic acid, 8.1 mM of propionic acid, 3.4 mM of butyric acid, and 1 mM each of valeric, isovaleric, isobutyric, and 2-methylbutyric acids. D-Glucose (20%, wt/vol), DL-lactate sodium salt (10%, vol/vol), DL-malate disodium salt (10%, wt/vol), and succinate disodium salt (10%, wt/vol) were prepared as separate anaerobic solutions under O₂-free CO₂ and autoclaved. These solutions were added to the medium to achieve the final concentrations shown in the figures.

In some cases, mixed ruminal bacteria were added to batch culture fermentations. Ruminal contents were collected from a 500-kg ruminally fistulated Holstein steer fed 29 kg of sorghum silage supplemented with 2.5 kg of concentrate supplement once daily with ad libitum access to hay. The ruminal contents were obtained 1.5 h after feeding and strained through four layers of cheesecloth into an Erlenmeyer flask with an O₂-free CO₂ headspace. The flask was not disturbed for 30 min while being incubated at 39°C in a water bath, permitting feed particles to rise to the top of the flask. Mixed ruminal bacteria were obtained via a two-step centrifugation procedure. Particle-free ruminal fluid was anaerobically transferred into centrifuge bottles as described above and subjected to a low-speed centrifugation (30 × g, 30 min, 25°C) to sediment protozoa and particulate matter. The supernatant was then centrifuged (6,000 × g, 30 min, 25°C) again, and the supernatant was discarded. The cell pellet was washed once with 10 ml of anaerobic basal medium (10,000 × g, 15 min, 25°C) and resuspended in 10 ml of anaerobic medium.

**Culture conditions.** Batch culture fermentations (500 ml) were conducted in gas washing bottles that were modified to remove or add samples through a butyl rubber stopper. The bottles were constantly purged with O₂-free CO₂ and placed in a 39°C water bath and were periodically mixed. Continuous culture experiments were performed with a model F-1000 fermentor (New Brunswick Scientific Co., Edison, N.J.) that had a modified 360-ml chemostat vessel for the culture of strictly anaerobic bacteria (25). The medium used for growing strain HD4 in continuous culture was identical to that described previously for use with batch culture studies except that valerate was the only volatile fatty acid added and resazurin was not used. DL-Lactate and DL-malate concentrations used are shown in Table 1. The dilution rate was 0.05 h⁻¹. The extracellular pH was...
TABLE 1. Effect of extracellular pH, lactate concentration, and malate on growth of *S. ruminantium* HD4 in continuous culture (dilution rate, 0.05 h⁻¹)

<table>
<thead>
<tr>
<th>Extracellular pH</th>
<th>Substrate concn (mM)</th>
<th>Residual substrate concn (mM)</th>
<th>OD₆₀₀</th>
<th>Protein (mg/liter)</th>
<th>Carbohydrate (mg/liter)</th>
<th>Succinate (mM)</th>
<th>Acetate (mM)</th>
<th>Propionate (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8</td>
<td>Lactate 6</td>
<td>Malate 0</td>
<td>Lactate 3.0 (50)</td>
<td>Malate 0.1</td>
<td>0.121</td>
<td>31.9</td>
<td>17.7</td>
<td>0.1</td>
</tr>
<tr>
<td>6.8</td>
<td>Lactate 35</td>
<td>Malate 0</td>
<td>Lactate 18.1 (48)</td>
<td>Malate 0.1</td>
<td>0.322</td>
<td>59.8</td>
<td>23.9</td>
<td>0.3</td>
</tr>
<tr>
<td>6.8</td>
<td>Lactate 60</td>
<td>Malate 0</td>
<td>Lactate 44.6 (35)</td>
<td>Malate 0.1</td>
<td>0.351</td>
<td>64.5</td>
<td>27.1</td>
<td>0</td>
</tr>
<tr>
<td>5.5</td>
<td>Lactate 6</td>
<td>Malate 0</td>
<td>Lactate 8.8 (70)</td>
<td>Malate 0.2</td>
<td>0.489</td>
<td>80.9</td>
<td>60.2</td>
<td>0.1</td>
</tr>
<tr>
<td>5.5</td>
<td>Lactate 54</td>
<td>Malate 0</td>
<td>Lactate 32.1 (40)</td>
<td>Malate 0</td>
<td>0.514</td>
<td>95.0</td>
<td>35.6</td>
<td>0.1</td>
</tr>
<tr>
<td>5.5</td>
<td>Lactate 6</td>
<td>Malate 8</td>
<td>Lactate 1.1 (80)</td>
<td>Malate 3.2 (62)</td>
<td>0.320</td>
<td>59.1</td>
<td>35.3</td>
<td>3.6</td>
</tr>
<tr>
<td>5.5</td>
<td>Lactate 28</td>
<td>Malate 8</td>
<td>Lactate 6.3 (78)</td>
<td>Malate 4.0 (51)</td>
<td>0.624</td>
<td>154</td>
<td>54.4</td>
<td>3.7</td>
</tr>
<tr>
<td>5.5</td>
<td>Lactate 57</td>
<td>Malate 8</td>
<td>Lactate 12.8 (77)</td>
<td>Malate 2.8 (64)</td>
<td>0.726</td>
<td>138.0</td>
<td>66.8</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Values in parentheses represent percent substrate utilized.

**Sampling and analysis.** Samples (50 ml) were removed from the continuous culture vessel with a syringe, and samples (22 ml) were collected through the butyl rubber stopper of each gas washing bottle with a syringe and needle. All samples were immediately centrifuged (10,000 × g, 15 min, 4°C), and the cell pellets were washed once and resuspended in deionized H₂O. Both the cell pellet and the culture supernatant were stored at −20°C.

**Bacterial growth.** Bacterial growth was monitored by measuring optical density at 600 nm (OD₆₀₀) with a spectrophotometer. An OD₆₀₀ of less than 0.1 was considered to represent bacterial washout for continuous culture samples. Glucose concentrations were measured by a coupled enzyme assay (2). Volatile fatty acids (VFA) were measured by gas-liquid chromatography with a GC-14A (Shimadzu Scientific Instruments, Columbia, Md.) gas chromatograph (column temperature, 125°C; injector temperature, 170°C; detector temperature, 175°C) equipped with an autosampler and GP 10% SP-1200–1% H₃PO₄ 80/100 mesh size Chromosorb W AW column (Supelco, Bellefonte, Pa.). (1). VFA concentrations were corrected for exogenous VFA present in the growth medium. Malate, succinate, and lactate were quantified by high-pressure liquid chromatography with an organic acid column (16). Cellular carbohydrate was determined with the anthrone reagent (11). Protein from 0.2 N NaOH-hydrolyzed cells (100°C, 15 min) or cell extracts was determined by the method of Lowry et al. (15) and compared with a bovine serum albumin standard.

**Results and Discussion.**

**Batch culture studies.** When *S. ruminantium* HD4 was grown in medium that contained d-lactate, acetate and propionate were the primary end products with little accumulation of malate or succinate (Fig. 1a). These results are consistent with the known fermentation pathways utilized by *S. ruminantium* (9, 20). In the presence of d-lactate and d-malate, strain HD4 catabolized both organic acids for the initial 20 h (Fig. 1b). During this time, there were increases in acetate and propionate concentrations (data not shown). Another end product resulting from the lactate-plus-malate fermentation was succinate. Production of this acid by *S. ruminantium* HD4 was previously reported (19, 20), and it can account for as much as 41% of the total recoverable carbon at high dilution rates in a glucose-limited chemostat (19). At 20 h, succinate and malate reached near-equimolar concentrations (~7 mM) and malate utilization ceased. Lactate utilization continued, as did acetate and propionate accumulation (data not shown), but at slower rates than those exhibited during malate utilization. It should be noted that *S. ruminantium* is unable to grow on malate in the absence of lactate or extracellular H₂ (16, 21).

Strain H18 in the presence of d-lactate and d-malate exhibited fermentation similar to that of strain HD4 (Fig. 1c). Lactate and malate utilization occurred simultaneously for the initial 24 h. During that time, acetate, propionate (data not shown), and succinate concentrations increased. As observed with strain HD4 (Fig. 1b), malate and succinate reached near-equimolar concentrations (~7 mM) and malate utilization ceased at 24 h (Fig. 1c). In contrast to the corresponding fermentation by strain HD4, no further utilization of lactate occurred after malate utilization was inhibited. Because *S. ruminantium* H18 has a strict dicarboxylic acid requirement for growth on lactate (30), it is likely that the cessation of malate utilization inhibited growth on lactate.

The reason for the accumulation of succinate by both strains of *S. ruminantium* in the presence of malate is unclear. Previously, production of this acid by *S. ruminantium* HD4 has been reported during growth in glucose-limited or ammonia-limited continuous culture and by lactate-grown cells of *S. ruminantium* H18 in batch culture (19, 20, 30, 33). At a dilution rate of 0.2 h⁻¹ in glucose-limited continuous culture, succinate accounted for as much as 41% of the total recoverable carbon and the highest production of succinate coincided with maximal growth yields of *S. ruminantium* HD4 (19, 20). The authors hypothesized that a site for energy conservation may exist somewhere in the pathway of succinate formation, a site independent of the reactions leading to propionate formation, such as succinate efflux.

To further investigate the effect of succinate on lactate-plus-malate utilization, 17 mM succinate was added to the lactate-plus-malate fermentation of both *S. ruminantium* strains at 14 h (Fig. 2). When 17 mM succinate was added to the lactate-plus-malate fermentation of strain HD4 at 14 h, little malate was fermented and no succinate was utilized over the next 41 h (Fig. 2a). However, lactate was utilized over the next 22 h. Malate utilization ceased at near-equimolar concentrations of malate and succinate (~17 mM). Addition of 17 mM succinate at 14 h to the lactate-plus-malate fermentation by strain H18 yielded results different from those observed for strain HD4 (Fig. 2b). Malate and lactate continued to be utilized after succinate addition up to 20 h. After 20 h, little malate was utilized and the rate of lactate utilization decreased. No succinate was utilized by strain H18. Collectively, these results...
suggest that succinate alters malate utilization by these \textit{S. ruminantium} strains.

It has been reported that \textit{S. ruminantium} H18 decarboxylates succinate 25-fold faster than strain HD4, and strain H18 is capable of decarboxylating succinate in the presence of lactate (30). It should be noted that in this study strain H18 was grown in medium that contained aspartate rather than malate (30). Therefore, it appears that cells grown in aspartate-plus-lactate medium may be more capable of decarboxylating succinate than cells cultured in malate-plus-lactate medium. Because clarified ruminal fluid can serve as a source of vitamins and unidentified growth factors, 20% clarified ruminal fluid was added to the fermentation of DL-lactate plus DL-malate by both \textit{S. ruminantium} strains. However, succinate still accumulated in the medium (data not shown).

Previous research with \textit{Bacillus subtilis}, \textit{Escherichia coli}, and a \textit{Pseudomonas} sp. showed that succinate transport was inhibited by DL-malate in all three bacteria (18). Furthermore, a single transport system is responsible for the uptake of L-malate and succinate in \textit{B. subtilis} (8). Inhibition of malate utilization by succinate in \textit{S. ruminantium} might also be associated with transport of both organic acids by the same transport system. However, even though several attempts were made to measure malate uptake in \textit{S. ruminantium}, we were not successful.

Experiments were conducted to evaluate the effects of adding mixed ruminal bacteria to the lactate-plus-malate fermentation by strain H18 (Fig. 3). Prior to the mixed-bacterium inoculation at 30 h, lactate and malate utilization by strain H18 resulted in the concomitant production of acetate, propionate, and succinate as well as inhibition of lactate and malate utilization at 18 h. Following the addition of mixed ruminal bacteria to the fermentation at 30 h, succinate, lactate, and malate were utilized and there were increases in acetate and propionate concentrations. Even though these results do not exclude the possibility that \textit{S. ruminantium} strains are responsible

![FIG. 1. Fermentation products of \textit{S. ruminantium} during growth on DL-lactate with and without the addition of DL-malate. (a) Strain HD4 on DL-lactate; (b) strain HD4 on DL-lactate and DL-malate; and (c) strain H18 on DL-lactate with DL-malate. • acetate; ▲ propionate; □ malate; ■ succinate; and △ lactate. All values represent the means of duplicate incubations.](http://aem.asm.org/)

![FIG. 2. Fermentation products of (a) \textit{S. ruminantium} HD4 and (b) \textit{S. ruminantium} H18 during growth on DL-lactate plus DL-malate with addition of 17 mM succinate at 14 h of incubation. • malate; ■ succinate; and △ lactate. All values represent the means of duplicate incubations.](http://aem.asm.org/)
for succinate decarboxylation within the rumen, they do suggest that other ruminal bacteria or the mixed population may be more active in this capacity than either \textit{S. ruminantium} strain alone. In addition, they suggest that the mixed bacterial population may provide growth factors not provided in our medium that are required to decarboxylate succinate.

\textit{S. ruminantium} strains decarboxylate succinate to yield propionate and have been implicated as being primarily responsible for succinate decarboxylation within the rumen (27). Other members of the genus are also capable of succinate decarboxylation. A newly isolated species, \textit{Selenomonas acidaminovorans}, is able to grow solely on succinate, and its decarboxylation of this organic acid is thought to result in a sodium efflux and formation of a sodium gradient (10). However, organisms even more active in succinate decarboxylation seem to exist in the rumen. \textit{Schwartzia succinivorans} and \textit{Succinicladi-}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig3}
\caption{Fermentation products of \textit{S. ruminantium} H18 during growth on \textit{d}-lactate and \textit{d}-malate with mixed ruminal bacteria (10 ml) added at 30 h (indicated by the arrow). \textcircled{O}, acetate; \textbullet{}, propionate; \textsquare{}, malate; \textblacksquare{}, succinate; and \texttriangle{}, lactate. All values represent the means of duplicate incubations.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.8\textwidth]{fig4}
\caption{Fermentation products of \textit{S. ruminantium} during growth on \textit{d}-glucose with or without \textit{d}-malate. (a) Strain HD4 on glucose; (b) strain H18 on glucose; (c) strain HD4 on glucose and malate; and (d) strain H18 on glucose and malate. \textcircled{O}, acetate; \textbullet{}, propionate; \textsquare{}, malate; \textblacksquare{}, succinate; \texttriangle{}, lactate; and \textblacksquare{}, glucose. All values represent the means of duplicate incubations.}
\end{figure}

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To determine the effects of other carbon sources on malate utilization, \textit{S. ruminantium} HD4 and H18 were grown in batch culture on glucose (Fig. 4). After a 2- to 4-h lag period, glucose was rapidly fermented (within 8 h) by both strains, and lactate was the primary end product (Fig. 4a and b). Rates of glucose utilization were similar for both strains. After glucose was depleted, lactate was utilized by both strains and resulted in acetate and propionate accumulation. This sequential utilization of substrates, which results in a diauxic growth pattern for \textit{S. ruminantium}, is well documented (3, 19, 24, 28). Lactate utilization by \textit{S. ruminantium} HD4 (Fig. 4a) proceeded at a much higher rate than that for strain H18 (Fig. 4b). Therefore, the strain HD4 fermentation resulted in higher concentrations of acetate and propionate. The inability of strain H18 to ferment lactate as rapidly as strain HD4 is due to its strict re-
quirement for aspartate, malate, or fumarate (30). Very little malate or succinate accumulated throughout either fermentation, and similar growth patterns were noted for both strains, with peak growth (OD$_{600}$, 1.6) coinciding with the depletion of glucose at 8 h.

Malate was not utilized by either strain in the presence of glucose (Fig. 4c and d). After glucose was depleted, malate was fermented to a greater extent by strain H18 than by strain HD4. Even though little malate was utilized by strain HD4, lactate was utilized between 8 and 24 h (Fig. 4c). Little lactate was fermented by strain H18 (Fig. 4d). Growth of strain HD4 on glucose plus malate was similar to that reported above for strain HD4 on glucose (data not shown). Although strain H18 exhibited a lower growth rate compared to that on glucose only ($k = 0.28$ h$^{-1}$ versus $k = 0.39$ h$^{-1}$), growth on glucose plus malate reached similar peak values (OD$_{600}$, 1.6). Previously, it has been reported that during growth of *S. ruminantium* HD4 on glucose the addition of L-aspartate had little effect on the growth rate (14).

When both strains of *S. ruminantium* were grown on glucose with 10 mM succinate added at 2 h, succinate concentrations did not change in the presence of glucose (data not shown). Recent research has shown that succinate decarboxylation by *S. ruminantium* is inhibited in the presence of glucose, and our data agrees with this finding (30). However, after glucose was depleted and lactate was being utilized as the growth substrate, no further change in succinate concentration occurred for either strain. This contradicts earlier findings for strain H18, in which succinate decarboxylation occurred after glucose depletion (30). The fermentation patterns for each strain were similar to those reported earlier for growth on glucose.

**Continuous culture studies.** In general, the dilution rate within the rumen is between 0.05 and 0.10 h$^{-1}$ (12). Most studies aimed at evaluating lactate utilization by *S. ruminantium* have been conducted in batch culture, and few experiments have been performed in continuous culture. Therefore, experiments were conducted to examine the effect of extracellular pH, lactate concentration, and malate addition on the growth of *S. ruminantium* HD4 in continuous culture (Table 1). When *S. ruminantium* HD4 was grown on lactate at pH 6.8, the primary end products were acetate and propionate with all concentrations of lactate. Little succinate or malate was produced. Even though not all lactate was utilized, as the lactate concentration was increased there were corresponding increases in OD$_{600}$ protein, and carbohydrate. These results suggested that lactate was limiting growth. Lactate utilization ranged between 35 and 50%. *S. ruminantium* HD4 was unable to grow on 6 mM lactate at an extracellular pH of 5.5 (Table 1). Growth did occur on 30 and 54 mM lactate at this pH, and acetate and propionate were the main end products that were produced. Little malate or succinate accumulated. Bacterial protein and OD$_{600}$ increased as lactate concentration increased, and there was a decrease in cellular carbohydrate. When 8 mM malate was added to the growth medium, strain HD4 was able to grow on 6 mM lactate at pH 5.5 and 80% of the lactate was utilized. Acetate, propionate, and succinate were the primary fermentation products produced with all three lactate concentrations in the presence of malate. The addition of malate increased the amount of lactate utilized and the OD$_{600}$, as well as the concentrations of protein and cellular carbohydrate synthesized by strain HD4. Lactate utilization ranged between 77 and 80% in the presence of malate compared to 40 and 70% in its absence. Malate utilization ranged between 51 and 64%. Similar effects were seen when strain HD4 was grown at a dilution rate of 0.10 h$^{-1}$ (data not shown).

When domestic ruminants (beef and dairy cattle) are fed diets high in rapidly fermentable carbohydrates (i.e., cereal grains), lactate can accumulate and decrease ruminal pH (26, 27, 29). Lactate concentrations as high as 29 mM have been observed with these types of diets (7). If lactate concentrations remain elevated, ruminal pH will drop below 6.0, and this leads to a variety of microbial and physiological problems (e.g., decreased fiber digestion, decreased digesta turnover, decreased salivation, rumen ulceration, founder, and death) (26, 29).

Based on our continuous culture results, it appears that malate enhances the ability of strain HD4 to grow on all three lactate concentrations at an extracellular pH of 5.5 (Table 1). These results are consistent with the observation that malate treatment increased final pH and decreased lactate concentrations in mixed ruminal microorganism fermentations of cracked corn and soluble starch (5, 17). Therefore, by adding malate to the diets of ruminants fed high levels of rapidly fermentable carbohydrates, it may be possible to improve the ability of *S. ruminantium* HD4 to utilize lactate at pHs of ≤6.0. In addition to commercial sources, forages, such as alfalfa and Bermuda grass, are potential sources of malate that could be included in ruminant diets (6).

**Conclusions.** Our results show that succinate accumulates in the medium when two strains of *S. ruminantium* ferment lactate plus malate. It is unclear why this bacterium produces succinate rather than decarboxylating all of the succinate to propionate. However, when mixed ruminal bacteria were added, succinate was utilized fairly rapidly (Fig. 3). These results suggest that succinate will not accumulate in the rumen if malate is present. *S. ruminantium* HD4 was unable to grow on 6 mM lactate at extracellular pH 5.5 in continuous culture, but addition of 8 mM malate to the medium allowed growth at this pH. Therefore, adding malate to the diets of ruminants (beef and dairy cattle) fed high levels of cereal grains may improve the ability of *S. ruminantium* to ferment lactate when the ruminal pH drops below 6.0.

**ACKNOWLEDGMENT**

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**REFERENCES**