Lysine Degradation by Ruminal *Fusobacterium necrophorum*

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Summary

Three experiments were conducted to characterize lysine fermentation by *Fusobacterium necrophorum*, a ruminal bacterium that is known to degrade amino acids. In Experiment 1, 7 strains of *Fusobacterium necrophorum* were inoculated into media containing lysine (50 m*M*), lactate (50 m*M*), or lysine plus lactate (50 m*M* each) as the major energy substrate to evaluate growth and ammonia production. All strains grew with lysine, lactate, or lactate plus lysine as the primary substrate. When grown with lysine, all strains produced ammonia as an end product, even if lactate was also present. Smaller concentrations of ammonia for medium containing lactate plus lysine when compared with lysine alone indicate that the *Fusobacterium* strains used lactate as a growth substrate that stimulated utilization of ammonia. In Experiment 2, the 2 strains tested were able to degrade extensively both lysine and glutamic acid. Some evidence was detected for partial utilization for growth of histidine, methionine, and tryptophan by strain A21. In Experiment 3, the minimum inhibitory concentration (**MIC**) of the antibiotic tylosin was 25 μg/mL when *Fusobacterium necrophorum* strains A21 and B35 were grown in either lysine or lactate-enriched medium. The MIC of monensin was 6.25 and 3.9 μg/mL for strains A21 and B35, respectively, when grown in lysine-enriched medium, but > 50 and 10.9 μg/mL when the strains were grown in lactate-enriched medium. These findings may lead to ways that ruminal lysine degradation may be controlled.

Key words: bacterial digestion, lactate, lysine degradation

Introduction

High milk-producing dairy cows require a well balanced supply of amino acids. Lysine is often a limiting amino acid for dairy cows, especially when they are fed diets containing large amounts of corn proteins. Degradation of amino acids by ruminal microorganisms, however, makes it impossible to increase lysine supply to the cow by simply adding more lysine to the diet. Therefore, much research has been conducted to find ways to protect lysine from ruminal degradation.

Besides protecting lysine from ruminal degradation, altering the ability of ruminal microbes to degrade lysine might also be possible. A better understanding of lysine degradation by ruminal microorganisms could lead to opportunities to control lysine degradation in the rumen. In general, amino acids in the rumen are degraded by bacteria that are classified as hyper-ammonia producing bacteria, which exist in small numbers in the rumen but nonetheless produce large amounts of ammonia by degradation of amino acids. *Fusobacterium necrophorum* has been identified as a hyper-ammonia producing bacterium. It has been isolated from rumen fluid enriched with lysine as a growth substrate, suggesting that *Fusobacterium necrophorum* may be one of the major bacteria that contributes to lysine degradation in the rumen.

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Our objectives were to: 1) identify the ability of ruminal *Fusobacterium necrophorum* to degrade lysine with or without the presence of lactate; 2) study the ability of *Fusobacterium necrophorum* to degrade a range of amino acids; and 3) evaluate the minimum inhibitory concentration (**MIC**) of tylosin and monensin against *Fusobacterium necrophorum* strains when their growth media contained lysine or lactate as the major energy source.

Experimental Procedures

General methods. Basal medium for growing *Fusobacterium* contained 292 mg K_2HPO_4, 292 KH_2PO_4, 480 mg $(NH_4)_2SO_4$, 480 mg NaCl, 100 mg $MgSO_4 \times 7\ H_2O$, 64 mg $CaCl_2 \times 2\ H_2O$, 1 g trypticase, and 0.5 g yeast extract dissolved in deionized water to a final volume of 1 L. *Fusobacterium necrophorum* strains were grown anaerobically in blood agar plates. A single colony of each strain was inoculated into anaerobic brain heart infusion broth supplemented with cysteine hydrochloride and incubated. Then, samples of each culture tube were inoculated into brain heart infusion broth and incubated before being diluted and used for inoculation.

Experiment 1. Media were enriched with lysine (50 m*M*), lactate (50 m*M*), or lactate plus lysine (50 m*M* each) by dissolving in the basal medium and adjusting to pH 7.0. Seven strains of *Fusobacterium necrophorum* (A21, A27, A29, B33, B34, B35, and B36) were inoculated into the enriched media and incubated for 48 hours at 37°C. Optical density was measured every 12 hours to measure growth, and samples for ammonia analysis were collected at the end of the experiments by acidifying culture media with *m*-phosphoric acid.

Experiment 2. Basal media preparations were enriched with 50 m*M* of the amino acids L-tryptophan, L-alanine, L-glutamic acid, L-methionine, L-histidine, or L-lysine. Media were inoculated with ruminal *Fusobacterium necrophorum* strains A21 or B35.

Experiment 3. Ruminal *Fusobacterium necrophorum* strains A21 and B35 were used for MIC determination. Stock solutions of tylosin and monensin contained 1 mg/mL of each antibiotic. The MIC was determined by using 96-well plates with concentrations of each antibiotic ranging from 50 to 0.097 μg/mL. The MIC was determined as the least concentration of antibiotic at which no bacterial growth was observed.

Results and Discussion

Experiment 1. All strains of ruminal *Fusobacterium necrophorum* had the ability to grow in media enriched with lysine, lactate, or lactate plus lysine. *Fusobacterium necrophorum* strains grown in medium containing lysine produced more ($P < 0.01$) ammonia than those receiving only lactate, indicating the degradation of the lysine (Table 1). The smaller concentration of ammonia for lactate plus lysine than for lysine alone is likely reflective of greater uptake of ammonia as a consequence of greater bacterial growth. The previous conclusion is supported by estimates of growth based on optical density (data not shown). These results demonstrate that ruminal *Fusobacterium necrophorum* has the ability to use lysine as the sole energy source, which agrees with the general characteristics of other *Fusobacterium* species reported by others.

Experiment 2. Results of ammonia concentrations (Table 2) and optical density values (data not shown) demonstrated that ruminal *Fusobacterium necrophorum* strains A21 and B35 degraded glutamic acid and lysine nearly completely by 48 hours of incubation. Some evidence supported partial utilization for growth of some other amino acids such as histidine, methionine, and tryptophan for strain A21. To date, however, little research exists supporting the

ability of ruminal strains of *Fusobacterium necrophorum* to degrade amino acids and use them as the sole energy source.

Experiment 3. The MIC for tylosin was 25 µg/mL for both *Fusobacterium necrophorum* strains A21 and B35 whether they were grown in lactate or lysine-enriched media (Table 3). Thus, the sensitivity of *Fusobacterium necrophorum* to tylosin seems to be independent of growth substrate.

When they were grown in the lactate-enriched medium, the MIC for monensin was more than 50 µg/mL for strain A21 and 10.9 µg/mL for strain B35. In contrast, when they were grown in the lysine-enriched medium, the MIC for monensin was 6.25 µg/mL for strain A21 and 3.9 µg/mL for strain B35, demonstrating that both strains were much more sensitive to the antibiotic effects of monensin when growing on lysine than when growing on lactate (Table 3). Ruminal *Fusobacterium necrophorum* is a gram negative bacterium, but some research showed that its growth could be inhibited by monensin at concentrations of 5 µM. Previous research found that monensin could reduce the transport (uptake) of lysine by bacteria, which could explain our results.

In general, our results indicate that tylosin and monensin may reduce *Fusobacterium necrophorum* in the rumen, and hence might control lysine degradation in the rumen and make it more available for the animal.

Table 1. Ammonia concentration (m*M*) after 48 hours of incubation of various *Fusobacterium necrophorum* strains in media enriched with lactate, lysine, or lactate plus lysine

	Medium contents[1]		
Strain	Lactate[c]	Lysine[a]	Lactate + lysine[b]
A21	7.5	97.0	68.7
A27	11.8	94.8	54.1
A29	12.2	96.4	52.7
B33	11.9	72.0	40.7
B34	11.8	71.7	37.6
B35	8.5	98.0	47.1
B36	13.2	80.0	45.0

[a,b,c] Media enrichments not bearing a common letter differ ($P < 0.01$). Media enrichments and strain affected ($P \leq 0.05$) ammonia concentration, but their interaction was not significant.
[1] Largest SEM among treatments = 9.6.

Table 2. Ammonia concentration (m*M*) after 48 hours of incubation of *Fusobacterium necrophrum* strains in media enriched with various amino acids

		Amino acid[1]					
Strain	Control[c,d]	Lys[a]	Ala[d,e]	Trp[e,f]	Met[f]	His[c]	Glu[b]
A21	12.2	91.5	10.9	6.6	6.4	17.0	51.0
B35	10.7	81.4	8.3	6.0	4.3	11.4	49.7

[a,b,c,d,e,f] Amino acids not bearing a common letter differ ($P < 0.05$). Amino acid and strain affected ($P < 0.01$) ammonia concentration, but their interaction was not significant.
[1] Lys = lysine; Ala = alanine; Trp = tryptophan; Met = methionine; His = histidine; Glu = glutamic acid. Largest SEM among treatments = 2.5.

Table 3. Minimal inhibitory concentration of tylosin and monensin on *Fusobacterium necrophorum* strains in lysine- or lactate-enriched media

		Minimal inhibitory concentration (μg/mL)	
Strain	Media enrichment	Tylosin	Monensin
A21	Lysine	25	6.25
A21	Lactate	25	>50
B35	Lysine	25	3.9
B35	Lactate	25	10.9