

# Antimicrobial activity of select anti-methanogenic nitro- and thio-containing chemicals

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## Introduction and Objectives

- New technologies are needed to help livestock producers maintain optimal health and wellbeing in their animals while minimizing risks of propagating and disseminating antimicrobial resistant bacteria to human or animal populations.
- Where possible, these interventions should contribute to the efficiency, profitability and environmental sustainability of animal production so as to avoid passing higher costs on to the consumer.
- Methane production within the rumen results in the loss of 2-12% of the gross energy consumed by the host, costing the U.S. cattle feeding industry as much as \$350,000 to \$700,000/day.
- Rumen methanogenesis also contributes 20% of the U.S. emission of this greenhouse gas.
- The objectives of this experiment were to examine the antimicrobial activity of potential anti-methanogenic chemicals to see if their applications may be combined to yield technologies economically more acceptable for livestock producers.

## Results

Table 1. Ethyl nitroacetate was a more potent inhibitor of rumen methane production than  $\alpha$ -lipoic acid.

Treatment	Gas produced (mL)	Hydrogen produced ( $\mu\text{mol/mL}$ )	Methane produced ( $\mu\text{mol/mL}$ )
No added inhibitor	9.00	0.15	19.09
3 mM Ethyl nitroacetate	7.00	1.89*	0.52*
9 mM Ethyl nitroacetate	7.67	2.71*	0.45*
3 mM $\alpha$ -Lipoic acid	10.50	0.16	18.65
9 mM $\alpha$ -Lipoic acid	8.67	0.09*	16.20*
Combined (each at 3 mM)	6.67	2.55*	0.31*
Combined (each at 9 mM)	5.50	1.64	0.29
<b>P value</b>	<b>0.0180</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>
<b>SEM</b>	<b>0.852</b>	<b>0.263</b>	<b>0.426</b>

\*Means differ from controls at  $P < 0.05$ .

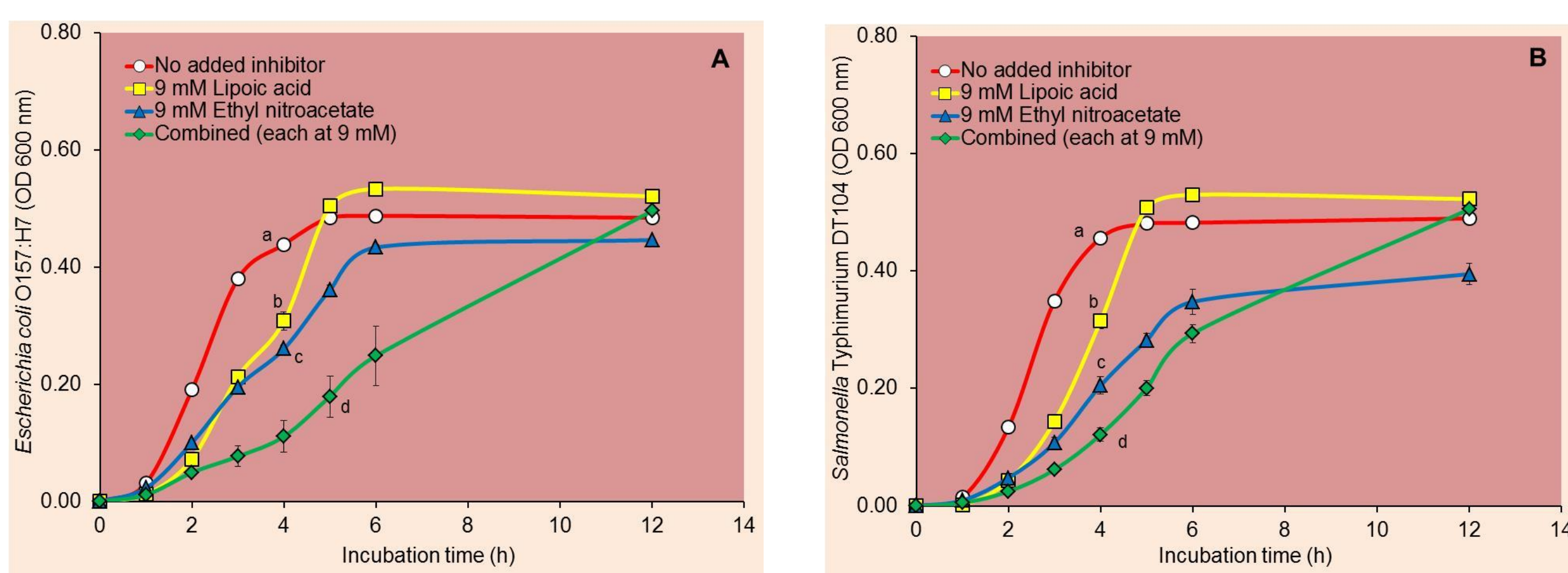


Figure 1. Ethyl nitroacetate was more potent in inhibiting growth rates of *E. coli* O157:H7 (A) and *S. Typhimurium* DT104 (B) than  $\alpha$ -lipoic acid when assessed during log phase growth in buffered tryptic soy broth, although evidence of synergy was apparent when both compounds were administered together. Curves with unlike letters differ at  $P < 0.05$ .

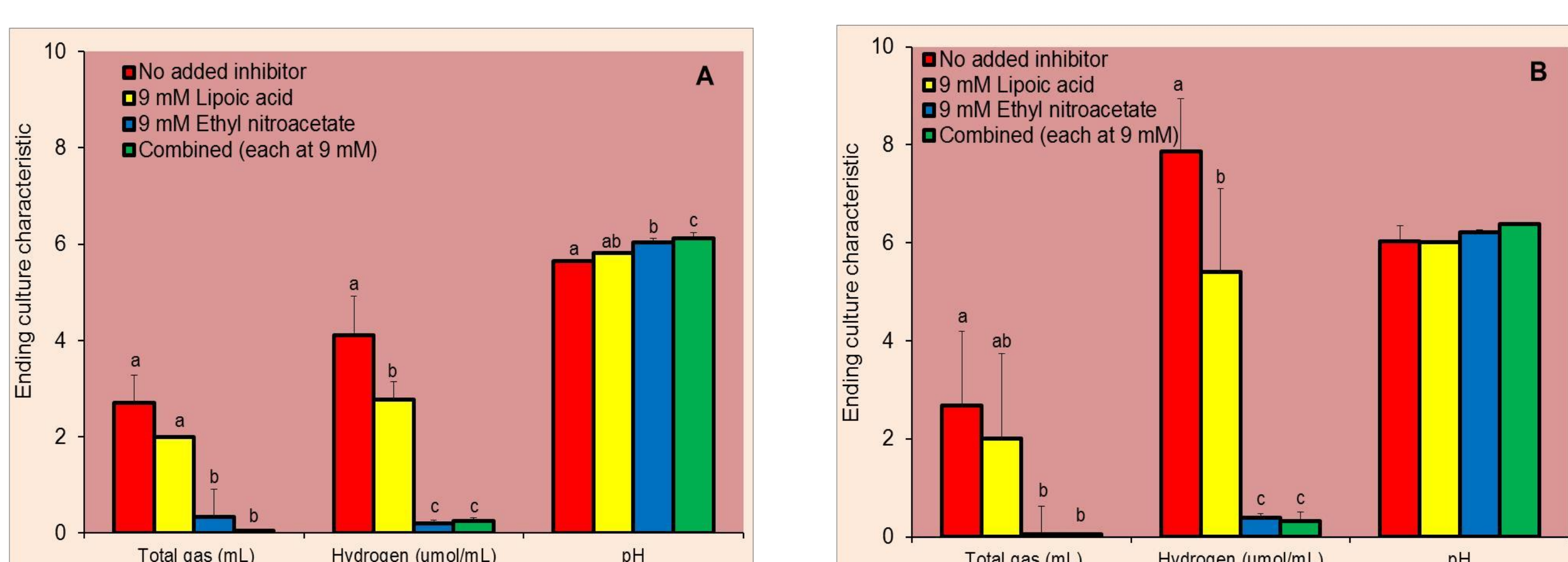


Figure 2. Ethyl nitroacetate was more potent than  $\alpha$ -lipoic in inhibiting hydrogen evolution by *S. Typhimurium* DT104 when grown in buffered tryptic soy broth without (A) or with supplemental formate (B). Means with unlike letters differ at  $P < 0.05$ .

## Materials and Methods

### Bacteria, growth and assay conditions

*Escherichia coli* O157:H7 strain 933, *Salmonella* Typhimurium DT104 and *S. Typhimurium* NVSL 9501776 were grown anaerobically in buffered (pH 6.8) tryptic soy broth supplemented without or with nitro- or sulfur-containing compounds (3 or 9 mM) and with or without 16 mM sodium formate as indicated. When used, chlorate was added to achieve 5 mM. Freshly collected rumen contents were cultured anaerobically for 24 h at 39°C with 0.2 g alfalfa forage and without or with nitro- and sulfur-containing compounds at 3 or 9 mM. For washed cell assays, *E. coli* O157:H7 cells were harvested from overnight cultures grown without or with 10 mM fumaric acid, washed and then resuspended in anaerobic buffer. Assays were conducted with cells, 4 mL/vial, incubated at 37°C for 1.5 h with 60 mM formate or with 30 mM hydrogen and 20 mM fumaric acid.

### Growth, total gas, methane and hydrogen measurements

Growth was measured via optical density at 600 nm or by viable cell counts. Total gas produced was measured by volume displacement. Methane and hydrogen were measured by gas chromatography.

### Statistical analysis

Cultures and assays were incubated in triplicate. Tests for treatment effects were conducted using a general analysis of variance with a Dunnett's comparison to controls or an LSD multiple comparison of means.

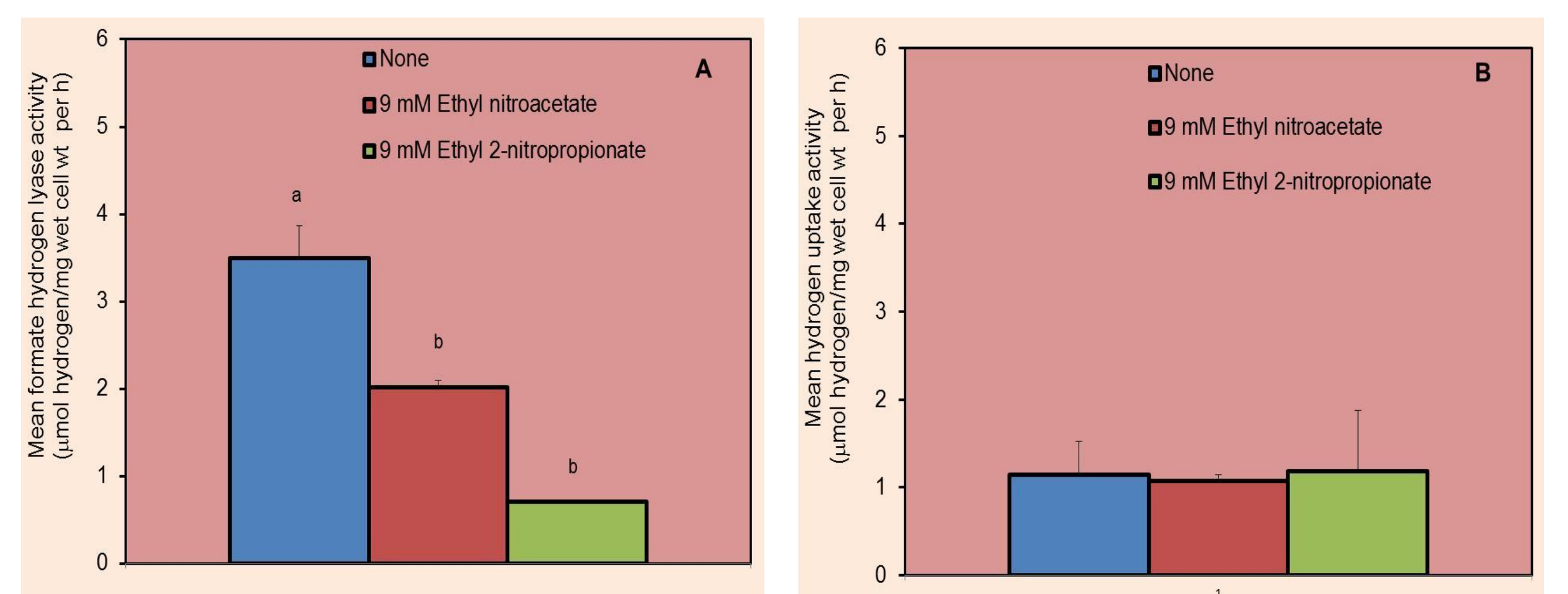


Figure 3. Ethyl nitroacetate and a related compound, ethyl 2-nitropropionate, inhibited ( $P < 0.05$ ) hydrogenase-catalyzed hydrogen evolution activity from formate (4A) but not hydrogenase-catalyzed uptake of hydrogen for fumaric acid reduction (4B) by washed cells of *E. coli* O157:H7.

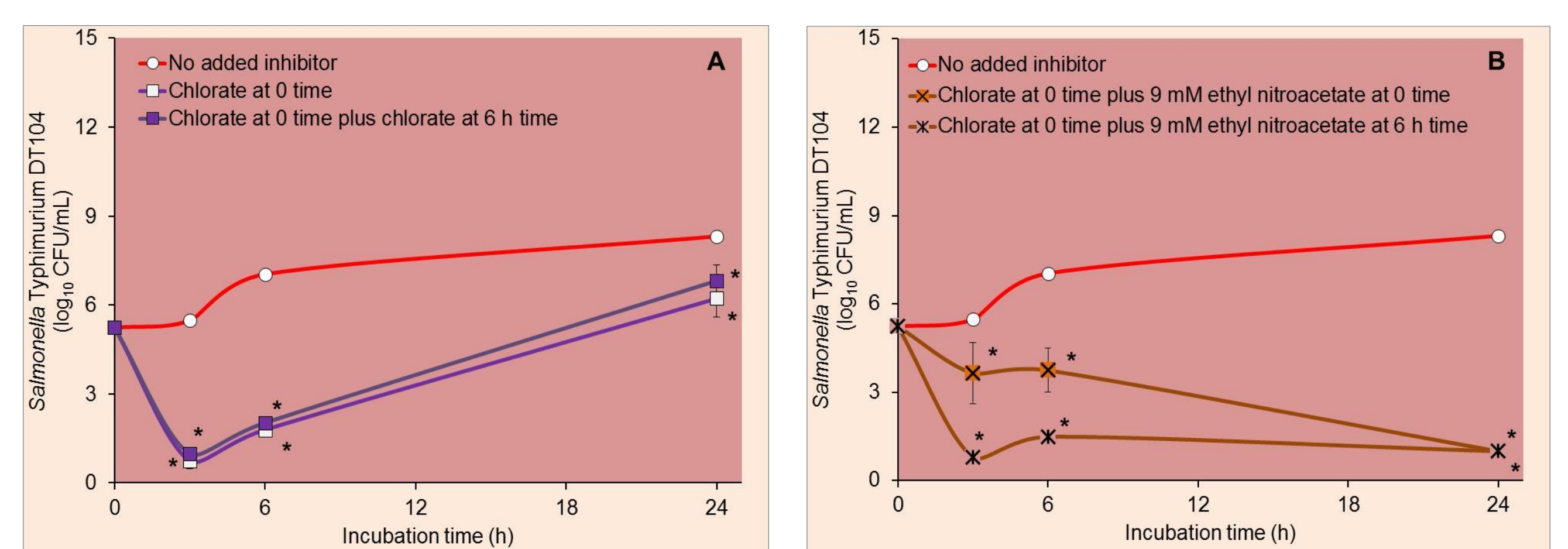


Figure 4. Adaptation of *S. Typhimurium* DT104 to the bactericidal effect of chlorate that commonly occurs during pure culture in buffered tryptic soy broth (A) was effectively overcome by co-administration of ethyl nitroacetate (B). Means with asterisks differ ( $P < 0.05$ ) from control values at each respective time point.

## Implications

Ethyl nitroacetate inhibited ruminal methane production and potentially could be used by producers to reduce economic and environmental costs of this digestive inefficiency.

Ethyl nitroacetate exhibited antimicrobial activity against *E. coli* O157:H7 and *S. Typhimurium* DT104 via disruption of important hydrogen- and formate-metabolizing activities.

Ethyl nitroacetate synergistically enhanced the bactericidal activity of chlorate against *S. Typhimurium* DT104, possibly by disrupting compensatory hydrogenase-catalyzed evolution of hydrogen employed by the bacterium to redirect electron flow away from the reduction of chlorate to cytotoxic chlorite.

Future studies are warranted to determine if ethyl nitroacetate or related nitrocompounds can reduce fitness and virulence of *E. coli* O157:H7 and *S. Typhimurium* DT104 in the gut of food producing animals and their production environments.

## Acknowledgements

The expert technical assistance of Emily Northcliffe is greatly appreciated.