Inhibitory Effect of Select Nitrocompounds on Growth and Survivability of *Listeria monocytogenes* In Vitro†‡

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ABSTRACT

We report the effects of 2-nitro-1-propanol (2NPOH), 2-nitroethanol (2NEOH), and nitroethane (NE) on growth and survivability of *Listeria monocytogenes*. In all cases, inhibition was greatest with 2NPOH and least with NE. For example, specific growth rates of *L. monocytogenes* strain 18 declined (P < 0.05) 76, 60, and 29% from controls during aerobic culture at 37°C in brain heart infusion broth containing 10 mM 2NPOH, 2NEOH, or NE, respectively. Mean specific growth rate for the controls incubated likewise without added nitrocompound was 0.62 ± 0.02 h⁻¹. Specific growth rates of *L. monocytogenes* Scott A decreased (P < 0.05) 67, 45, and 11%, respectively, from controls (0.67 ± 0.02 h⁻¹) when cultured similarly. Specific growth rates for *L. monocytogenes* strain 18 incubated similarly except at 30°C were reduced (P < 0.05) 76, 60, and 30%, respectively, and were reduced (P < 0.05) 78, 23, and 23% during anaerobic culture at 30°C in brain heart infusion broth containing 15 mM 2NPOH, 2NEOH, or NE (control rates ranged from 0.37 ± 0.07 to 0.74 ± 0.05 h⁻¹). Survivability of *L. monocytogenes* strain 18 was reduced (P < 0.05) during aerobic storage (4 months at 4°C) in brain heart infusion broth containing 2NPOH or 2NEOH (by 7.8 and 1.9 log units, respectively) but not NE. The inhibitory effect of 2NPOH was approximately 20% greater during growth at pH 7.0 than at pH 5.6 or 8.0. These results demonstrate the differential inhibitory activity of 2NPOH, 2NEOH, and NE against *L. monocytogenes* in vitro.

*L. monocytogenes* is an important pathogen capable of causing invasive infections in humans and animals (11, 13). This organism is widely distributed in nature and has been isolated from natural and food-processing environments as well as from numerous animal species (12, 14, 35). *L. monocytogenes* is not fastidious and is capable of growing in minimal media from <1.0 up to 50°C and at pH values of between 4.6 and 9.6 (11, 29). Human listeriosis has resulted in a number of serious foodborne outbreaks characterized by high rates of morbidity and mortality for susceptible individuals (13, 31). It is estimated that *L. monocytogenes* causes approximately 2,500 cases of serious human illness and numerous deaths per year in the United States, leading to substantial medical costs (27, 33). There is also evidence of listerial infections being manifested as febrile gastroenteritis (13). Because of the recognized potential of *L. monocytogenes* to contaminate raw and processed dairy products and ready-to-eat foods (7, 8, 19, 30), numerous chemical and physical interventions have been developed to control it in processing environments (22). Nevertheless, processors continue to be under pressure to find and develop new methods and additives to prevent contamination (32, 34).

Recent work with 2-nitro-1-propanol (2NPOH) has shown that this compound exhibits broad-spectrum antimicrobial activity, inhibiting the growth of *Salmonella enterica* serovar Typhimurium, *Escherichia coli* O157:H7, and *Enterococcus faecalis* in vitro (17) and reducing gut concentrations of *Salmonella Typhimurium* in broilers (18). Research has also demonstrated that the synthetic nitrocompounds 2NPOH, nitroethanol (2NEOH), and nitroethane (NE) inhibit the growth of *Salmonella Typhimurium* in vitro (3) and inhibit methane production by ruminal contents of cattle (2). Further evidence exists for 2NPOH and 2NEOH inhibition of uric acid–degrading bacteria (20). The objective of this study was to examine the effect of 2NPOH, 2NEOH, and NE on maximal specific growth rates and survivability of *L. monocytogenes* in vitro.

MATERIALS AND METHODS

**Bacterial strains.** *L. monocytogenes* strain 18 (serotype 1/2a) and Scott A (serotype 4b) were provided by Dr. J. F. Frank, University of Georgia. *Listeria innocua* ATCC strain 51742 was obtained from the American Type Culture Collection.

**Culture conditions and experimental design.** The effects of 2NPOH, 2NEOH, or NE on specific growth rates of *L. monocytogenes* were determined during incubation (30 or 37°C as indicated) of tubes containing aerobic or anaerobic (under 90% N₂–5% CO₂–5% H₂) brain heart infusion (BHI) broth (Becton, Dick-
inson and Company, Sparks, Md.) medium supplemented with or without the respective nitrocompounds as indicated. Supplements were added to the media immediately before inoculation as small volumes from filter-sterilized (0.2-μm Acrodisc syringe filter, Pall Life Sciences, Ann Arbor, Mich.) 150 mM stock solutions of the respective test compounds. The nitrocompounds were purchased from Sigma-Aldrich (St. Louis, Mo.); stock solutions were prepared in distilled water and all tubes were brought to final volume by additions of distilled water. Growth was measured as the change in optical density at 600 nm with a Spectronic 20D spectrophotometer (Spectronic, Rochester, N.Y.). The effect of pH on the inhibitory activity of 2NPOH was determined during incubation (30°C) of tubes containing 2NPOH supplemented (10 mM) BHI broth that had been adjusted to pH 5.6, 7.0, or 8.0. Tubes were inoculated with 0.2 ml of a culture grown overnight at 30°C in BHI broth lacking any added nitrocompound. The effects of 2NPOH, 2NEOH, or NE on survivability of L. monocytogenes incubated at 4°C were determined following extended (4 months) incubation of tubes containing aerobic BHI broth supplemented as indicated and inoculated with 7.7 log CFU ml⁻¹ of the overnight grown culture. Initial and final cell concentrations were determined via quantitative recovery on modified Oxford agar (Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK) that was incubated for 24 h at 30°C. All cultures were incubated without agitation. To determine if nitrocompound concentrations decreased during incubation (37°C) of uninoculated and inoculated (with L. monocytogenes strain 18) BHI medium, samples collected at 0 and 24 h from tubes containing medium supplemented with 10 mM 2NPOH, 2NEOH, or NE were analyzed colorimetrically to measure nitrocompound concentrations (24).

**Statistical analysis.** Tests were conducted in triplicate. Specific growth rates or mean concentrations (CFU per milliliter) of L. monocytogenes were analyzed for treatment differences by analysis of variance and least significant difference separation of means.

**RESULTS**

When L. monocytogenes was cultured aerobically at 30°C with 5, 10, or 15 mM added 2NPOH, specific growth rates were reduced (P < 0.05) 55, 76, and 88% from rates of control cultures grown without added 2NPOH (0.42 ± 0.02 h⁻¹) (Fig. 1). When L. monocytogenes was cultured with 5, 10, or 15 mM added 2NEOH, specific growth rates were reduced (P < 0.05) 37, 60, and 62% from rates of control cultures grown without added 2NEOH (0.52 ± 0.05 h⁻¹) (Fig. 1). When L. monocytogenes was cultured with 10 or 15 mM added NE, specific growth rates were reduced (P < 0.05) 30 and 35% from the rate of the controls (0.37 ± 0.07 h⁻¹); however, the specific growth rate during culture in medium containing 5 mM NE (0.37 ± 0.07 h⁻¹) was not significantly reduced from the controls (Fig. 1).

Specific growth rates of L. monocytogenes strain 18 were reduced (P < 0.05) 58, 33, and 8% during aerobic culture at 37°C in BHI broth containing 10 mM added 2NPOH, 2NEOH, or NE, respectively, from rates of control cultures grown without added nitrocompound (0.62 ± 0.02 h⁻¹) (Fig. 2). When L. monocytogenes Scott A was cultured likewise, specific growth rates were reduced (P < 0.05) 67, 45, and 11%, respectively, from rates of control cultures (0.62 ± 0.02 h⁻¹) (Fig. 2). Specific growth rates of L. innocua ATCC 51742 cultured likewise in BHI with 10 mM added 2NPOH and 2NEOH were reduced (P < 0.05) 65 and 33%, respectively, from rates of control cultures (0.54 ± 0.03 h⁻¹), but growth rates were not reduced during culture with 10 mM added NE (Fig. 2). All nitrocompound concentrations decreased by 21 to 34% from initial levels (10 mM) following 24 h of incubation (37°C) of BHI medium. These decreases in nitrocompound concentrations were observed in BHI medium in the presence or absence of L. monocytogenes.

When L. monocytogenes strain 18 was cultured anaerobically with 10 or 15 mM added 2NPOH, specific growth rates were reduced (P < 0.05) 36 and 78% from rates of control cultures grown without added 2NPOH (0.70 ± 0.02 h⁻¹) (Fig. 3). When L. monocytogenes strain 18 was cultured anaerobically with 15 mM 2NEOH or NE, specific growth rates were reduced 18 and 23%, respectively, from
controls incubated without either nitrocompound (0.70 ± 0.01 h⁻¹) (Fig. 3). Specific growth rates of *L. monocytogenes* strain 18 were not reduced when cultured anaerobically with 10 mM 2NEOH or NE (Fig. 3).

During *L. monocytogenes* strain 18 incubation in BHI containing no added nitrocompound, the specific growth rate was more rapid (*P* < 0.05) at pH 8.0 than at pH 5.6, with controls incubated at pH 7.0 being intermediate (Fig. 4). The inhibitory effect of 2NPOH was approximately 20% greater during aerobic growth at pH 7.0 than at pH 5.6 or 8.0 (Fig. 4). When *L. monocytogenes* strain 18 was inoculated with 7.7 log CFU ml⁻¹, survivability was reduced from that in controls by more than 3.9 log units during aerobic storage at 4°C for 120 days with 2NPOH but only 1.87 and 0.94 log units 10 mM 2NEOH or 15 mM NE, respectively (Fig. 5).

**DISCUSSION**

Results presented here show that 2NPOH, 2NEOH, and NE exhibit inhibitory activity against *L. monocytogenes* and *L. innocua* in vitro, with 2NPOH being the most effective. Whether the nitrocompounds may be used as disinfectants or as food or feed additives to control foodborne pathogens is not yet clear and ultimately will depend on whether these compounds are found to be safe and efficacious. Whereas aliphatic nitrocompounds are generally used as solvents and intermediates for organic synthesis, we have no knowledge that the nitrocompounds tested here are currently under review for use in foods or feeds. Secondary nitroalkanes such as 2-nitropropane and 2-nitrobutane cause damage to rat liver DNA and RNA and are mutagenic in their ionized form when tested by the Ames Salmonella assay (9, 10). However, primary nitroalkanes and nitrocarbinols such as 2NPOH were not carcinogenic or mutagenic when similarly tested (9, 10). Moreover, toxic effects were not observed in rats following chronic (2 year) inhalation exposure to 100 or 200 ppm nitroethane (15) and the oral 50% lethal dose (*LD₅₀*) of 2NPOH to chicks was found to be 1,300 mg/kg of body weight (18). By comparison, the *LD₅₀* of 3-nitro-1-propanol, a naturally occurring regiochemical isomer of 2NPOH, is 77 mg/kg of body weight when orally administered to rats (26). 3-Nitro-1-propanol and another related compound, 3-nitro-1-propionic acid, are important natural toxins found in certain forages (4). In the study of Jung et al. (18), oral administration of 13 mg of 2NPOH per bird resulted in >2 log unit reductions cecal *Salmonella* Typhimurium concentrations, thus demonstrating that this compound may have application in reducing foodborne pathogens in animals.

Whereas animal infections by *L. monocytogenes* are common and can sometimes result in listeriosis or the establishment of carrier animals that shed high concentrations of the pathogen into the environment (28, 35), little work has been done to develop on-farm interventions for this pathogen. Ruminants appear to be particularly well suited
to harboring Listeria, with sheep and goats apparently more susceptible to disease than cattle, which conversely appear better suited to serve as natural reservoirs for this pathogen (21).

The use of the nitrocompounds as feed additives to control Listeria and Salmonella may have particular application to ruminants because they are also potent inhibitors of ruminal methanogenesis (1), a digestive inefficiency associated with ruminal energy metabolism that can result in losses of gross energy intake of 2 to 12% (16). Precedence exists for the experimental feeding of 2NPOH and NE to ruminants to inhibit ruminal methane-producing activity without any apparent adverse effects (2). Additionally, NE has been safely administered to cattle to enhance the ruminal degradation potential of 3-nitro-1-propanol (23, 24) and 2NPOH has been safely administered to cattle during comparative investigations of ruminal 2NPOH and 3-nitro-1-propanol metabolism (25). In ruminants, the various nitrocompounds would be presumed to be reduced to their respective amines as the related naturally occurring nitrocompounds, 3-nitro-1-propanol and 3-nitro-1-propionic acid, are reduced to 3-amino-1-propanol and -alanine, respectively, within populations of ruminal microbes (5). At present, Denitrobacterium detoxificans is the only known ruminal bacterium to possess appreciable ability to reduce 2NPOH, 2NEOH, NE, 3-nitro-1-propanol, and 3-nitro-1-propionic acid as well as several other nitrocompounds, using these as terminal electron acceptors for anaerobic respiration (6). In the present study, as much as 34% of the added 2NPOH or NE disappeared following 24 h of incubation at 37°C, presumably due to volatilization of these compounds and not to bacterial activity, as equivalent disappearance also occurred in sterile medium incubated concurrently.

In conclusion, results from the present study show that 2NEOH, NE, and, in particular, 2NPOH exhibited inhibitory activity against L. monocytogenes under a variety of conditions and suggest that these compounds may have potential to control Listeria in natural and man-made environments harboring the pathogen.

ACKNOWLEDGMENTS

We thank Ann Marie Prazak for her excellent technical assistance. Portions of this work were funded by the U.S. Department of Agriculture’s Borlaug Fellowship Program.

REFERENCES