

# *In Vitro* Comparison of Nitroethane, 2-Nitro-1-Propanol, Lauric Acid, Lauricidin<sup>®</sup> and the Hawaiian Marine Algae, *Chaetoceros* Activity Against Anaerobically Grown *Staphylococcus aureus*

Aleksandar K. Božić,<sup>1</sup>

Robin C. Anderson,<sup>2</sup>

Todd R. Callaway,<sup>2</sup>

David J. Nisbet,<sup>2</sup>

Steven C. Ricke,<sup>3,4</sup>

Philip G. Crandall,<sup>3</sup>

Corliss A. O'Bryan<sup>3\*</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, University of Novi Sad, Republic of Serbia

<sup>2</sup>Southern Plains Agricultural Research Center,

Food and Feed Safety<sup>3</sup> Research Center, College Station, Texas, USA

<sup>3</sup>Food Science Department and Center for Food Safety, University of Arkansas, Fayetteville, Arkansas, USA

<sup>4</sup>Poultry Science Department, University of Arkansas, Fayetteville, Arkansas, USA

\*Corresponding author:

Food Science University of Arkansas 2650 Young Ave.

Fayetteville, AR 72704 Ph: 479-575-5328 Fax: 479-575-3941

Email: cobryan@uark.edu

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## ABSTRACT

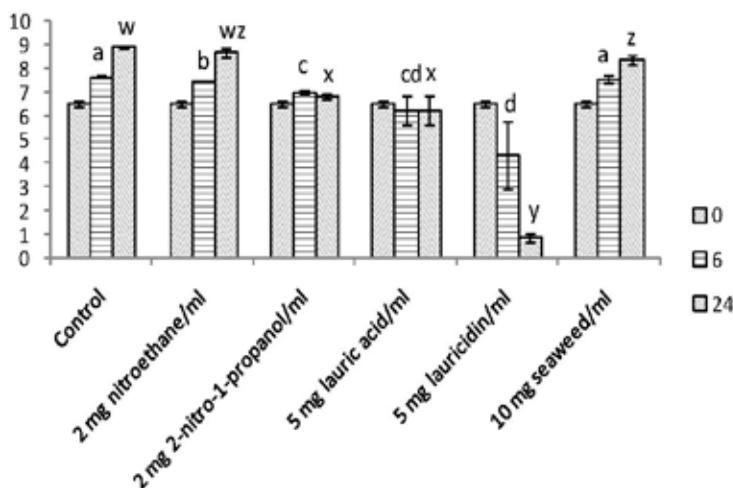
Mastitis is a common illness of dairy cattle and is very costly economically to the dairy farmer. Thus there is a need to develop broad-spectrum therapies that are effective while not leading to unacceptably long antibiotic withdrawal times. The effects of the CH<sub>4</sub>-inhibitors nitroethane (2 mg/ml), 2-nitro-1-propanol (2 mg/ml), lauric acid (5 mg/ml), the commercial product Lauricidin<sup>®</sup> (5 mg/ml), and a finely-ground product of the Hawaiian marine algae, *Chaetoceros* (10 mg/ml), were compared in pure cultures of *S. aureus*. Lauricidin<sup>®</sup> exhibited the most

bactericidal activity against *S. aureus*. These results suggest potential for treatments with a non antibiotic compound could be effective against mastitis.

## INTRODUCTION

One of the most common illnesses of dairy cattle and also one of the most economically devastating is mastitis, an infection of the udder.<sup>1</sup> Economic losses associated with mastitis have been estimated at 1.2 to 1.7 billion dollars per year in the United States alone.<sup>2</sup> *Staphylococcus aureus* is one of the most important bacteria that cause mastitis,<sup>3</sup> and may be present in the rumen.<sup>4</sup> *S. aureus* has developed increasing resistance to antibiotics commonly used to treat mastitis, including beta-lactam, aminoglycosides,

**Figure 1:** Effects of nitrocompounds, lauric acid, Lauricidin® or *Chaetoceros* on growth of *Staphylococcus aureus* in vitro. a-d Different superscripts within 6 hour time indicate significant difference ( $p < 0.05$ ). w-z Different superscripts within 24 hour time indicate significant difference ( $p < 0.05$ ).



fluoroquinolones, lincosamides, macrolides, and streptogramins.<sup>5-8</sup> This increase in antibiotic resistance coupled with contamination of milk with antibiotics is an emerging problem for the dairy industry.<sup>9</sup>

There is a need find other antimicrobials that may offer broader spectrum control measures against a wider range of problematic organisms. Nitrocompounds such as nitroethane, 2-nitroethanol, and 2-nitropropanol have been reported to be bacteriostatic to *Salmonella* and *Campylobacter* in the GI tract of pigs.<sup>10</sup> Lauric acid and other medium chain fatty acids have shown activity against Gram-positive bacteria and yeasts.<sup>11,12</sup> A monoglycerol ester of lauric acid named Lauricidin®, has been shown to have activity against pathogenic bacteria.<sup>13-15</sup> A highly unsaturated fatty acid, hexadecatrienoic acid, has been found in the marine algae *Chaetoceros*,<sup>16</sup> and has antibacterial activity against Gram-positive bacteria.<sup>17</sup>

The primary objective of the present study was to compare the antibacterial effects of nitroethane, 2-nitro-1-propanol, lauric acid, Lauricidin®, and the marine algae

*Chaetoceros* against *Staphylococcus aureus*.

## MATERIALS AND METHODS

### Bacterial strains and chemicals

*Staphylococcus aureus* was obtained from the culture collection of the Southern Plains Agricultural Research Center, ARS-USDA, College Station, Texas. Sodium laurate (sodium dodecanoate), nitroethane, and 2-nitro-1-propanol were purchased from Sigma-Aldrich (St. Louis, MO). Lauricidin® was provided by Dr Jon Kabara (Bradenton, FL, USA). The marine algae *Chaetoceros* was produced and harvested from an open continuous microalgae culture system at the Anuenue Fisheries Center, Sand Island, Oahu, Hawaii, and contained 2.46 mg hexadecatrienoic acid g<sup>-1</sup> of algae dry weight.

### Culture conditions

Pure cultures were grown in Brain Heart Infusion broth (BHI; Becton, Dickinson and Company, Sparks, MD, USA) prepared and distributed (10 ml per tube) anaerobically under 100% N<sub>2</sub> to 18 x 150 mm crimp top tubes. Sodium laurate, Lauricidin® (0.05 g each) and 0.10 gm of a ground product

(approximately 1 mm particle size) of the marine algae were added as dry additions to tubes before addition of medium. These tubes and those containing no prior additive were closed with rubber stoppers, crimped and sterilized by autoclaving. Nitroethane and 2-nitro-1-propanol were added to tubes containing sterilized medium as a filter sterilized (0.22 µm) concentrated stock solution. Nitroethane was prepared as a sodium salt as described by Majak et al<sup>19</sup> to increase its solubility in aqueous solutions. Upon inoculation with 0.2 ml of overnight cultures grown in unsupplemented anaerobic BHI, treated and control cultures were incubated upright without agitation at 39°C for 24 h. Because cultures containing lauric acid and Lauricidin® were opaque, and thus unsuitable for optical density determinations, viable cell counts were performed on all cultures to measure growth and survivability.

Fluid samples collected at indicated intervals were serially diluted (10-fold) in anaerobic (N<sub>2</sub>) phosphate buffered saline (pH 6.8) and spread to BHI agar plates (Becton, Dickinson and Company). Inoculated plates were incubated at 39°C in a Bactron Anaerobic Chamber (Sheldon Labs Manufacturing Inc., Cornelius, OR, USA) under an N<sub>2</sub>:CO<sub>2</sub>:H<sub>2</sub> (90:5:5) atmosphere for 24 h.

### Statistical analysis

Control and treated cultures were incubated in triplicate. Colony-forming units were transformed to log<sub>10</sub> and means were calculated. Differences were determined by Student's t test, with significance assigned at  $p < 0.05$ .

## RESULTS AND DISCUSSION

In this study, nitroethane and *Chaetoceros* had little effect on *S. aureus*. Lauric acid and 2-nitro-1-propanol suppressed growth slightly during the 24 hours of the test. Lauricidin® was the most successful compound tested in suppressing growth of *S. aureus* to less than 1 log at the 24 hour mark.

*S. aureus* is a major contributor to mastitis in dairy cattle, and is becoming increas-

ingly resistant to currently used antibiotics causing an emerging problem for the animal industry. Other antimicrobials with a broader spectrum of activity need to be developed. Fatty acids have been recognized historically as having antimicrobial activity against Gram-positive bacteria.<sup>13,20</sup> The mechanism of action of medium and long chain fatty acids against Gram-positive bacteria is thought to be disruption of cell membranes.<sup>21,22</sup> Fatty acid toxicity is apparently proportional to the degree of unsaturation.<sup>23</sup>

A glycerol monoester of lauric acid, Lauricidin®, has similar activity against gram-positive bacteria to that of lauric acid.<sup>13</sup> Dufour et al<sup>24</sup> found that Lauricidin® greatly increased the lag phase of *S. aureus* growth, and also kept the growth suppressed for 24 hours. Anang et al<sup>25</sup> found that Lauricidin® was more effective against the Gram-positive pathogen *L. monocytogenes* than against Gram-negatives such as *Salmonella* Enteritidis or *E. coli* O157:H7. Boddie and Nickerson<sup>26</sup> tested postmilking teat germicides containing Lauricidin® (1%), lactic acid (6%), and lauric acid (0.85%) against *S. aureus*, and reported numbers reduced by nearly 80%.

The marine algae *Chaetoceros* is known to contain the highly unsaturated fatty acid hexadecatrienoic acid.<sup>16</sup> Desbois et al.<sup>17</sup> reported that hexadecatrienoic acid found in cell extracts of the diatom *Phaeodactylum tricorutum* exhibited antibacterial activity against *Staphylococcus aureus*. In this study using the finely ground algae itself we found only a slight effect.

One solution to the global crisis of antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application. A new approach other than producing new, stronger antibiotics would be using the promising results from studies of fatty acids and monoglycerides.

## CONCLUSION

The primary objective of the present study was to compare the antibacterial effects of nitroethane, 2-nitro-1-propanol, lauric acid, Lauricidin®, and the marine algae *Chaetoc-*

eros against *S. aureus*. Lauricidin<sup>®</sup>, a glycerol monoester of lauric acid, was effective against *S. aureus*, reducing the level from the inoculums to below 1 log in 24 hours.

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