

# P1-96 Sensitivity of *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus*, and *Bacillus atrophaeus* to Elevated Hydrostatic Pressure in the Presence of Mild Heat, Nisin and Lysozyme

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## ABSTRACT

Commercial adoption of high-pressure processing is gaining momentum and industrial importance with recent advances in the engineering of pressure-based pasteurization units. The main curtailment of the technology is limited efficacy for inactivation of microbial spores. Current study investigated synergism of elevated hydrostatic pressure, mild heat, and two antimicrobials (nisin, and lysozyme) for inactivation of three spore suspensions. Various times (0, 3, 5, and 10 minutes) at pressure intensity level of 650 MPa (*e.g.* 94K PSI) of elevated hydrostatic pressure (Hub880 Explorer, Pressure BioScience Inc), were investigated at 50 °C for inactivation of *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus* (ATCC 7953), and *Bacillus atrophaeus* (ATCC 9372). The selected strains are currently considered as one of the most pressure-resistant natural isolates, the biological indicator for heat-based sterilization, and indicator for heat- and chemical-based decontamination interventions, respectively. The spore suspensions exposed to treatments at the above-mentioned intensity with and without the presence of Lysozyme (22.4 ml/L), and Nisin (5000 IU/ml) in HEPES buffer. The ANOVA conducted followed by LSD-based mean separation by OpenEpi software. Counts of *Bacillus amyloliquefaciens* were  $6.75 \pm 0.1$  prior to the treatment and were reduced to  $4.32 \pm 0.1$  after the treatment for 10 minutes at 650 MPa for 50 °C. These reductions were augmented in presence of nisin where the spore suspension was reduced by 2.21 Log CFU/mL. Nisin was similarly efficacious ( $P < 0.05$ ) for reducing *Bacillus atrophaeus* by 3.31 Log CFU/mL after the above-mentioned treatment for 10 minutes while was not capable of significant reductions ( $P \geq 0.05$ ) of *Geobacillus stearothermophilus*. Lysozyme, similarly, lead to 2.62 and 2.65 Log CFU/mL reductions of *Bacillus amyloliquefaciens*, and *Bacillus atrophaeus*, respectively. Results of current study indicate an optimized pressure-based intervention in presence of mild heat and antimicrobial agents could be efficacious for inactivation of >99% of microbial spores.

## Design, Materials and Methods

### Sporulation

- Cells from overnight culture were used
- Aliquots of 0.1 mL will be spread on Nutrient agar supplemented with  $10 \text{ mg L}^{-1} \text{ MnSO}_4 \cdot \text{H}_2\text{O}$
- The inoculated plates were incubated at 32 °C (*B. amyloliquefaciens*, *B. atrophaeus*) and 55 °C (*G. stearothermophilus*) for 3 days.
- The spore crop was harvested by flooding the plates with cold sterile deionized water thrice
- The final spore suspension in DIW was heat-shocked (80 °C, 15 min) and kept at 4 °C.

• **HPP treatment:** 0 – 650 Mpa, 50 °C

• **Antimicrobial compounds:** Lysozyme – 22.4 ml/L; Nisin – 5000 IU/ml

### Spore enumeration

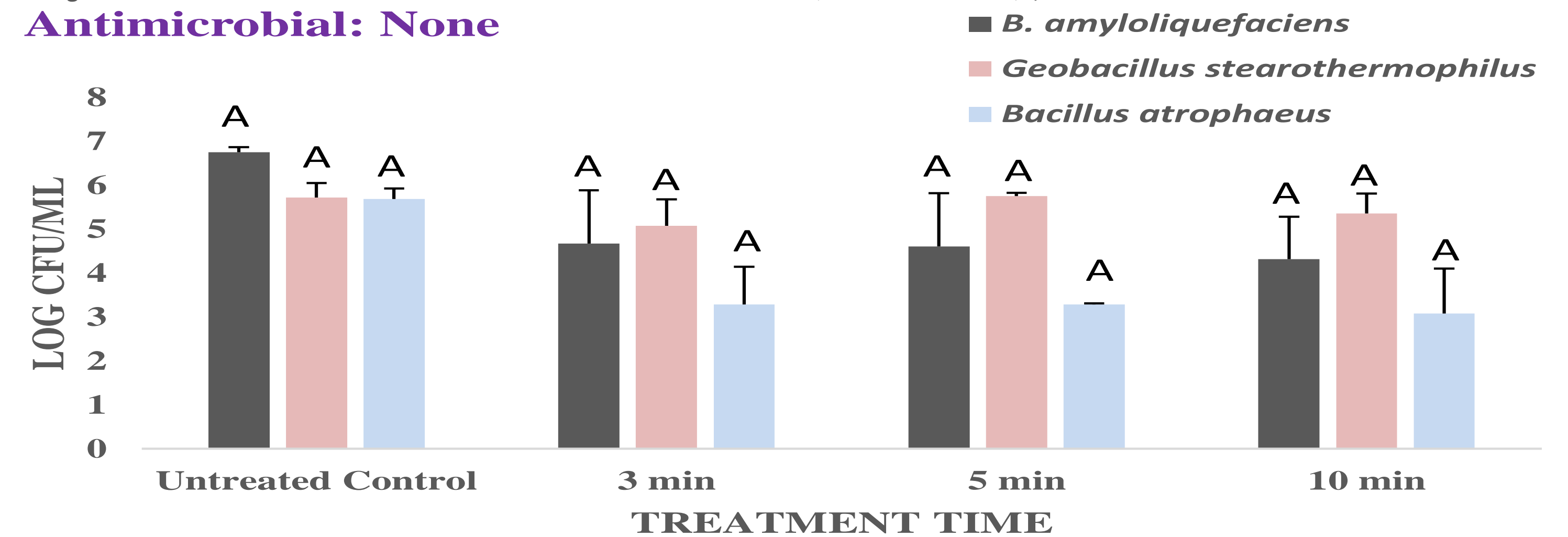
- Spore concentration before and after pressure-thermal and thermal processing will be determined by spread plating on TSAYE
- Samples were decimally diluted with 9 mL of 0.1 % MRD.
- Each tube dilution was mixed repeatedly using a high speed vortex mixer to yield a uniform spore suspension, and plated.
- The TSAYE plates were incubated aerobically for 72 h, until visible colonies are formed.

• Study was a **Randomized Complete Block Design** with:

- ✓ Two biologically independent repetitions (*i.e.*, two **blocking factor**)
- ✓ Each block, containing three **instrumental replications**
- ✓ Each instrumental replication consisting of two **microbiological replications**  
(Thus each presented value is mean of at least 12 repetitions)

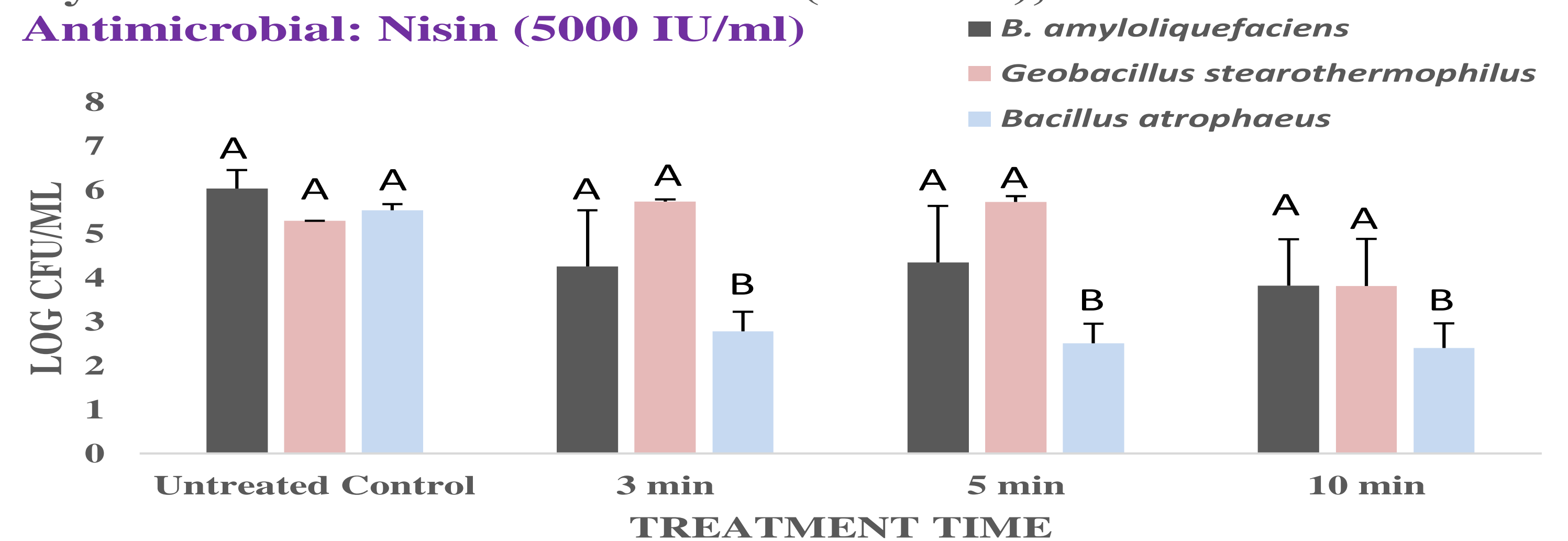
### Hydrostatic Pressure at 650 MPa (94K PSI), 50 °C

Antimicrobial: None



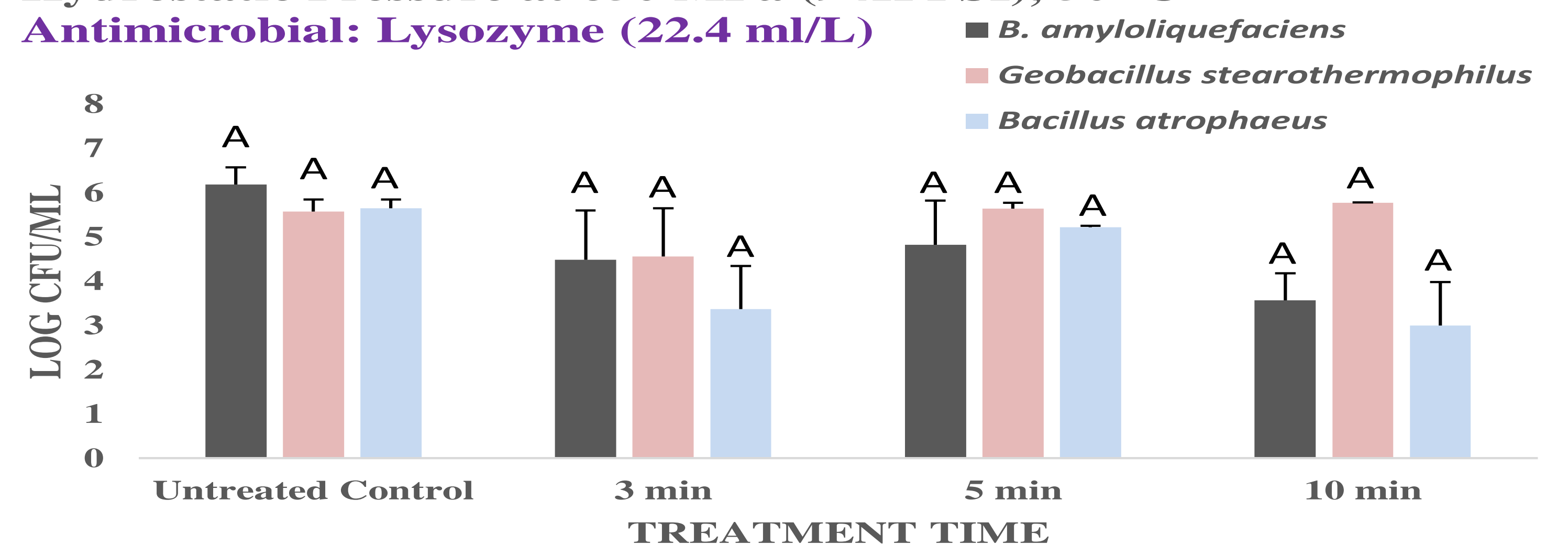
### Hydrostatic Pressure at 650 MPa (94K PSI), 50 °C

Antimicrobial: Nisin (5000 IU/ml)



### Hydrostatic Pressure at 650 MPa (94K PSI), 50 °C

Antimicrobial: Lysozyme (22.4 ml/L)



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