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# The Inhibitory Effect of *Bacillus subtilis* PB6 on *Salmonella enterica* serovars Heidelberg, Cerro and Uganda

## Introduction

*Salmonella enterica* serovars Heidelberg, Cerro and Uganda are associated with disease and mortality in dairy cattle with each shown to be resistant to many antibiotics.[1,2,3] The reduction in the use of antibiotics worldwide compels producers to look at alternative interventions to maintain not only the health of the animal but to provide safe food for the world's population. The use of direct-fed microbial (DFM) products is one alternative being used. While *in vitro* assays cannot mimic *in vivo* conditions, the work reported here demonstrated that the metabolites secreted by *Bacillus subtilis* PB6 (PB6), a direct-fed microbe, have the ability to inhibit the growth of these pathogens and may be a viable alternative to antibiotic use.

## Materials and Methods

### *Salmonella* isolates

*Salmonella enterica* serovar Heidelberg, *Salmonella enterica* serovar Cerro and *Salmonella enterica* serovar Uganda were provided by the Wisconsin Veterinary Diagnostic Laboratory (WVDL). Upon receipt, stock seeds of each organism were prepared and stored at -80°C.[4] In preparation for the inhibition assay, 0.1 mL of each *Salmonella* isolate was inoculated into 9.0 mL Tryptic Soy Broth (TSB, Difco, Franklin Lakes, NJ) and incubated without shaking at 37°C ± 2°C for 20-22 h.

### PB6 preparation

One gram of PB6 Concentrate was diluted in 9.0 mL 0.85% sterile saline and vortexed on high speed for three minutes. A further dilution in saline (1:9) was heated in a water bath at 80°C for 10 min. and immediately cooled in cold water to halt any further action of the heat. A 1.0 mL aliquot was dispensed into 99 mL of TSB and placed into a shaking incubator (150 rpm) at 37°C ± 2°C for 22-24 h.

### Cell free supernatant (CFS) preparation

Following incubation, 100 mL of the PB6 growth culture were centrifuged at room temperature at 5000 x g for 10 min. (Sovall® RC-5C Plus, Thermofisher Scientific, Asheville, NC). Approximately 10 mL of the supernatant were filter sterilized through a 0.22 µ filter and used the same day for the microtiter assay.

### Microtiter assay

The effect of the molecules secreted by PB6 on the growth of the *Salmonella* spp. isolates was evaluated in a microtiter plate assay (Molecular Devices, Sunnyvale, CA) measuring the optical density (OD) at 620 nm. In this experiment, the plate was read kinetically every 2 h over a 20 h period, while maintaining temperature at 37°C ± 2°C. Prior to each OD measurement, the plate was shaken for 5 sec. The results reflect the average OD measurements of four microtiter wells. A 100 µL aliquot of a test organism in TSB (1.0E+06 CFU (colony forming unit)/mL) and a 100 µL aliquot of CFS were dispensed into individual microtiter plate wells. Two negative controls were incorporated into the experiments: 1) 100 µL of test organism (1.0E+06 CFU/mL) and 100 µL of sterile media and 2) 100 µL of sterile media and 100 µL of sterile 0.85% saline.

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## Results& Discussion

In this experiment, metabolites secreted by PB6 demonstrated the ability to inhibit the growth of *Salmonella* ser. Heidelberg, *Salmonella* ser. Cerro and *Salmonella* ser. Uganda as noted by the lower OD readings compared to the corresponding positive controls. Biological systems can be highly variable, and while there are differences between the data sets, the commonality is that the growth of the challenge organisms was inhibited.

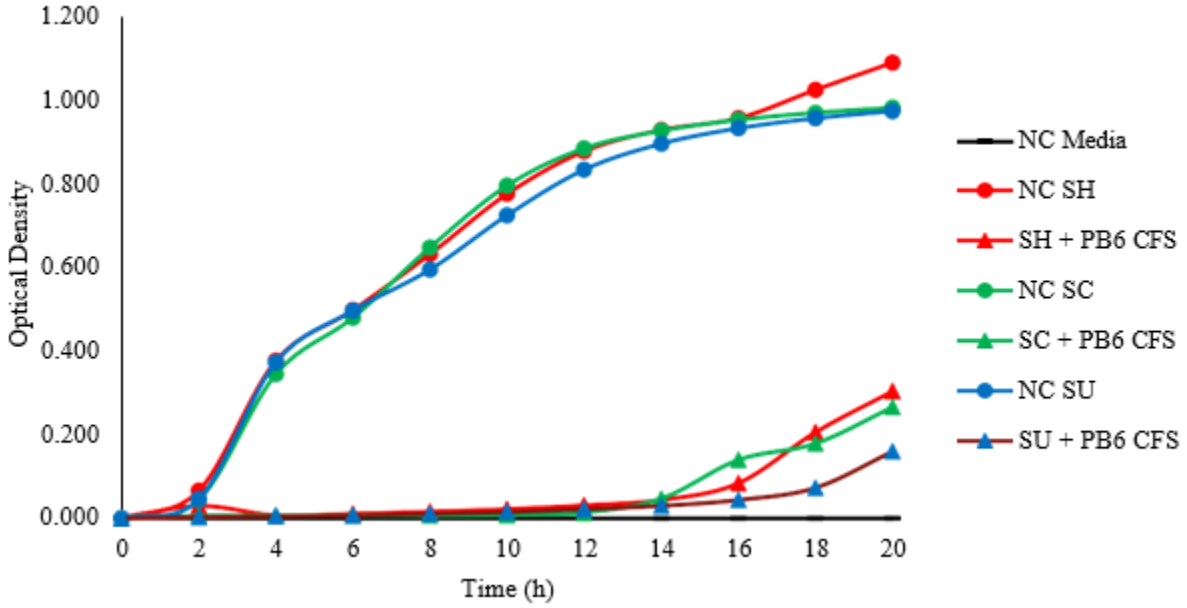


**Figure 1**. Effect of *Bacillus subtilis* PB6 (PB6) metabolites on the growth of *Salmonella* ser. Heidelberg (SH), *Salmonella* ser. Cerro (SC) and *Salmonella* ser. Uganda (SU) in a kinetic read assay (CFS = cell free supernatant).[5]

## Conclusion

The metabolites secreted by PB6 demonstrated the ability to inhibit the growth of three strains of *Salmonella* identified as major pathogens in the dairy industry. While this *in vitro* assay cannot mimic *in vivo* conditions, it does indicate that PB6 may be a viable alternative to antibiotics to support intestinal health. With the reduction in the use of antibiotics in animal agriculture, new strategies for raising healthy animals must be adopted if the safety of the world's food supply is to be maintained.

## References

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