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Antagonistic activity of bacteriocins produced by *Bacillus subtilis* against *Clostridium perfringens* type A

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Introduction

The purpose of this study was to evaluate different bacteriocins produced by various genotypes of *B. subtilis* against a variety of genotypically different isolates of *C. perfringens* type A. In a previous study evaluating the clostridial diversity in Midwest swine herds, we determined that there is a high degree of genetic diversity amongst *C. perfringens* type A (both between swine production systems as well as within individual sites) as determined by Random Amplified Polymorphic DNA (RAPD) PCR typing.[1]

Likewise, with *B. subtilis* there are numerous genotypes. These genetic differences yield variability in processes like sporulation, germination, cell wall synthesis, and antibiotic production.[2,3] The objective of this evaluation was to determine if bacteriocins produced by various genotypes of *B. subtilis* could inhibit the growth of various genotypes of *C. perfringens* type A.

Materials and methods

Six representative *C. perfringens* type A test strains were chosen from the six largest clusters created in a comprehensive dendrogram from Agtech Product's database. To date, this database represents well over two thousand isolates harvested from rectal swabs from numerous diverse swine production sites across the United States. These selected isolates were screened against bacteriocins produced by six different strains of *Bacillus subtilis*.

The six strains of *B. subtilis* were grown overnight in Tryptic Soy Broth (TSB) and the bacteriocin was harvested by centrifugation of the broth to remove the bacterial cells from the culture. The supernatant containing the bacteriocin was then filter sterilized to remove any remaining cells. The assays were set up in a 48 well plate to screen the six different bacteriocins against the six different *C. perfringens* type A isolates and included a positive and negative control. The positive control wells contained the BHI broth and were only inoculated with the clostridial target. Light absorbance readings, measured using a microplate reader, for the positive control wells were used to establish baseline 0% inhibition levels. The negative control wells contained only BHI broth and were used to establish the 100% inhibition levels. The test combinations of the various *B. subtilis* bacteriocins and the targeted *C. perfringens* type A wells contained 900µl of BHI broth, 100µl of the supernatant harvested from an overnight growth of the *Bacillus* strain, and 10µl of an overnight growth of the *Clostridium* isolate. Plates were incubated anaerobically for 24 hours and percent inhibition of clostridial growth by each of the bacteriocins was determined by light absorbance. This assay was done in duplicate and the average inhibition was reported.

Inhibition was calculated using the formula:

$$\frac{\text{(Positive Control Reading – Sample Reading)}}{\text{Positive Control Reading}} \times 100 = \%\ \text{Inhibition}$$

Results and discussion

Growth inhibition of the six *C. perfringens* type A strains by the various bacteriocins varied considerably – ranging from no inhibition (0%) to complete inhibition (100%) (Table 1). This variation across bacteriocin producers and targeted clostridial isolates may help explain the variable responses that may be observed with direct-fed microbial (DFM) supplementations. Just as sensitivity to various commercial antibiotics are observed against differing bacterial populations, differences in sensitivities are also noted between the bacteriocins produced by individual *B. subtilis* strains and between various clostridial isolates. Therefore, when designing a DFM for disease control strategies, the selection of strains to include should account for this variation.

References

[1]Baker A., et al. 2006. *Proc AD Leman Conf,*22(supp).
[2]Earl A., et al. 2007. *J Bacteriol* 189:1163-1170.
[3]Nakamura L. K., et al. 1999. *Int J Syst Bacteriol* 49:1211-1215.

Table 1: Percent inhibition of *C. perfringens* type A in response to six different *B. subtilis* bacteriocins

C. perfringens Isolate	Positive Control	Negative Control	*B. subtilis* #1	*B. subtilis* #2	*B. subtilis* #3	*B. subtilis* #4	*B. subtilis* #5	*B. subtilis* #6
CPA-1	0.00	100.00	0.00	100.00	99.82	97.31	96.69	100.00
CPA-2	0.00	100.00	4.06	100.00	98.11	22.91	96.29	99.15
CPA-3	0.00	100.00	0.99	99.66	99.43	96.32	97.58	98.69
CPA-4	0.00	100.00	8.42	100.00	100.00	70.51	93.15	95.40
CPA-5	0.00	100.00	13.41	100.00	98.81	94.47	94.14	94.65
CPA-6	0.00	100.00	4.09	98.60	98.63	96.01	96.12	96.12