COMPETITIVE INHIBITION OF MEAT SPOILAGE BACTERIA AND PATHOGENS

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Background

Brochothrix campestris was isolated from soil and grass and first described in detail by Talon and co-workers (1988). Subsequently, it was found to produce a broad spectrum bacteriocin (brochocin-C) inhibitory to a range of gram-positive bacteria including *Brochothrix thermosphacta*, *Listeria* spp. and lactic acid bacteria (Siragusa and Cutter, 1993). Purified brochocin-C was also active against vegetative cells and spores of *Bacillus* and *Clostridium* spp., including *C. botulinum* (McCormick *et al*., 1998).

The cost associated with the production of bacteriocins and inactivation in food environments often precludes their practical application as food preservatives. Thus, the use of viable, bacteriocin-producing cells as biopreservatives in meats is a reasonable alternative (Lücke, 2000). Unfortunately, it has been assumed that *B. campestris* would not be an acceptable strain for use in meats and research has been undertaken to heterologously express brochocin-C in "food grade" lactic acid bacteria such as *Carnobacterium piscicola* (McCormick *et al*., 1998; Garneau *et al*., 2003). This technique is tedious and it is difficult to develop strains in which the heterologous expression of brochocin-C is stable.

Preliminary, inoculation studies had shown that *B. campestris* could grow on pork adipose tissue at 4°C without producing undesirable odours or changes in the appearance of the fat. Also, there were no known published reports of the competitive inhibition of bacteria by *B. campestris* in broths. Thus, the current study was designed to provide needed data on the competitive effects of *B. campestris* co-inoculated with gram-positive spoilage bacteria and pathogens.

Objectives

This study was undertaken to determine the ability of *B. campestris* ATCC 43754 to control the growth of *B. thermosphacta* B2, *Lactobacillus sake* 1218 and *Listeria monocytogenes* when co-cultured in APT broth at 4°C.

Materials and methods

B. campestris ATCC 43754 was purchased from the American Type Culture Collection (Manassas, VA, USA). *L. sake* 1218 (Leisner *et al*., 1996) and *B. thermosphacta* B2 (Greer and Dilts, 2002) were meat spoilage strains. Two of the *L. monocytogenes* strains were isolated from a pork processing facility, one was a poultry isolate (ATCC 19111) and the fourth was a clinical isolate of *L. monocytogenes* Scott A. (Health Canada, Ottawa, ON). Inocula were prepared by growing the individual strains in APT broth (Difco, Becton Dickinson Co., Sparks, MD, USA) at 25°C for 18 h.

Growth studies were conducted in APT broth incubated at 4°C for 7 to 13 d. All experiments were conducted under aerobic conditions with the exception of mixed cultures with *L. sake* where growth was also determined under anaerobic conditions in a BBL anaerobic jar with 5-10% CO_2 (BBL Anaerobic System, Becton Dickinson). *B. campestris* ATCC 43754 was diluted to give an initial population of about 6 to 7 log CFU/ml and the target species were at initial numbers of 3 to 4 log CFU/ml. Prior to dilution, the 4 *L. monocytogenes* strains were mixed by combining equal volumes of the individual 18 h cultures.

The growth of the 3 target species (*B. thermosphacta* B2, *L. sake* 1218 or the 4 strain mixture of *L. monocytogenes*) was determined alone and in mixed culture with *B. campestris* ATCC 43754. To recover and enumerate *B. thermosphacta* during co-culture with *B. campestris* a modified MRS medium (Wilkinson and Jones, 1977) was utilized in the presence or absence of 0.1% potassium tellurite. *B. thermosphacta* B2 grows to produce black colonies on this medium while the growth of *B. campestris* ATCC 43754 is inhibited. Tryptone-phytone-yeast extract agar containing 0.02% erioglaucine (TPYE, Greer and Dilts,

1997) was used to enumerate *L. sake* 1218 during co-culture with *B. campestris* ATCC 43754. This medium would inhibit the growth of *B. campestris* ATCC 43754 while *L. sake* 1218 would produce pale green colonies. PALCAM-agar (Difco) was used to enumerate *L. monocytogenes* during co-culture with *B. campestris* ATCC 43754. *L. monocytogenes* produced black colonies on this medium (Corry *et al*., 1995) and *B. campestris* ATCC 43754 was inhibited. Streptomycin-thallous acetate-actidione agar (STAA, Corry *et al*., 1995) enabled the recovery of *B. campestris* ATCC 43754 while not permitting the growth of *L. sake* 1218 or *L. monocytogenes*. All media were inoculated using the spread plate technique with the exception of TPYE where Hydrophobic Grid Membrane Filtration was used.

Bacterial numbers were converted to common logarithms and reported as log CFU/ml. The significance of treatment differences was determined by analysis of variance using the general linear model procedures of the SAS Institute.

Results and discussion

Biopreservation of meats with bacteriocinogenic lactic acid bacteria (LAB) and their bacteriocins has been the subject of authoritative reviews (Stiles, 1996; Lücke, 2000). Although *B. campestris* ATCC 43754 was known to produce a broad spectrum bacteriocin (Siragusa and Cutter, 1993) the current study was the first to show that this organism could competitively restrict the growth of meat spoilage bacteria and pathogens in mixed cultures at 4°C (Tables 1 to 4) resulting in a significant reduction in bacterial numbers ($P < 0.05$).

The competitive strain, *B. campestris* ATCC 43754, increased by 2 log CFU/ml by about 5 d of incubation to reach a maximum population approximating 9 log CFU/ml. During co-culture with *B. thermosphacta* (Table 1) or *L. monocytogenes* (Table 2), *B. campestris* was bacteriostatic. This was evident in a reduction in the rate of growth of the target strains producing a 3-4 log CFU/ml reduction ($P < 0.05$) in bacterial numbers by the end of the incubation period. Lactobacilli have also been shown to inhibit the growth of *B. thermosphacta* (Shay *et al*., 1984) and *L. monocytogenes* (Schillinger *et al*., 1991) in broths and meat products.

In mixed culture with *L. sake* 1218 under aerobic conditions, *B. campestris* ATCC 43754 was initially bactericidal and *L. sake* could not be recovered from the culture media after 1 and 4 d of incubation (Table 3). Thereafter, *L. sake* 1218 began to grow but the numbers remained at least 4 log CFU/ml lower by the end of the incubation period when compared to those determined during the growth of *L. sake* 1218, alone (Table 3). Under anaerobic conditions, *B. campestris* ATCC 43754 demonstrated a prolonged bactericidal effect and no *L. sake* 1218 could be recovered from mixed cultures with *B. campestris* ATCC 43754 following 2 to 12 d of incubation at 4°C (Table 4), while *L. sake* 1218 alone grew to 9 log CFU/ml. Leisner *et al*. (1996) reported that *L. sake* 1218 could be inhibited in APT broth and in vacuum packaged beef by *Leuconostoc gelidum* UAL 187 and the inhibition of bacterial growth was of a sufficient magnitude to delay the sulphide spoilage by *L. sake* 1218.

Conclusions

The growth of *B. thermosphacta* B2, *L. sake* 1218 or a 4 strain mixture of *L. monocytogenes* was significantly inhibited in mixed culture with *B. campestris* ATCC 43754 during the incubation of APT broth at 4°C. Depending upon the target strain in the mixture and the gaseous atmosphere, the inhibition was either bacteriostatic or bactericidal. Additional research is necessary to transfer the system to meats and to insure that the competitive organism, *B. campestris* ATCC 43754, does not produce spoilage.

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Table 1. Aerobic growth of *B. thermosphacta* B2 in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*B. thermosphacta*	*B. thermosphacta* with *B. campestris*
0	7.04 ± 0.14	3.44 ± 0.24	2.25 ± 0.12
1	7.52 ± 0.33	3.14 ± 0.26	2.48 ± 0.40
2	8.16 ± 0.25	4.92 ± 0.07	3.01 ± 0.24
3	8.51 ± 0.28	5.68 ± 0.28	3.91 ± 0.13
4	8.69 ± 0.09	6.60 ± 0.04	4.01 ± 0.34
5	8.73 ± 0. 07	6.97 ± 0.12	4.32 ± 0.30
6	8.97 ± 0.08	6.97 ± 0.44	3.80 ± 0.40
7	8.90 ± 0.09	7.82 ± 0.15	4.28 ± 0.26

[1]Data are means and standard errors for 2 trials and 5 replications.

Table 2. Aerobic growth of a *L. monocytogenes* pool in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L.* monocytogenes[2]	*L. monocytogenes*[2] with *B. campestris*
0	6.90 ± 0.08	4.42 ± 0.05	4.45 ± 0.04
1	7.06 ± 0.09	4.64 ± 0.04	4.65 ± 0.04
3	8.44 ± 0.05	5.28 ± 0.07	5.34 ± 0.07
6	9.21 ± 0.09	6.36 ± 0.08	5.88 ± 0.14
9	9.10 ± 0.11	7.62 ± 0.09	5.92 ± 0.15
11	8.75 ± 0.18	8.68 ± 0.09	6.01 ± 0.08
13	8.45 ± 0.17	9.09 ± 0.04	5.80 ± 0.13

[1]Data are means and standard errors for 2 trials and 6 replications.
[2]Pool of 4 strains.

Table 3. Aerobic growth of *L. sake* 1218 in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L. sake*	*L. sake* with *B. campestris*
0	6.42 ± 0.05	3.22 ± 0.05	2.81 ± 0.33
1	6.49 ± 0.05	3.58 ± 0.11	0[2]
4	8.46 ± 0.14	6.18 ± 0.11	0
6	8.69 ± 0.02	5.91 ± 0.05	4.51 ± 0.18
8	8.81 ± 0.08	8.43 ± 0.11	3.91 ± 0.03

[1]Data are means and standard errors for 2 trials and 5 replications.
[2]<1 bacteria/ml.

Table 4. Anaerobic growth of *L. sake* 1218 in mixed culture with *B. campetris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L. sake*	*L. sake* with *B. campestris*
0	6.92 ± 0.03	2.91 ± 0.05	2.74 ± 0.06
2	7.68 ± 0.03	3.36 ± 0.01	0[2]
5	9.00 ± 0.04	5.87 ± 0.01	0
7	8.80 ± 0.03	6.29 ± 0.04	0
9	8.92 ± 0.03	8.04 ± 0.03	0
12	8.93 ± 0.07	9.21 ± 0.05	0

[1]Data are means and standard errors for 2 trials and 5 replications.
[2]<1 bacteria/ml.
Incubation was in a BBL anaerobic jar (Becton Dickinson) in an atmosphere containing 5-10% CO_2.