6-P12

CONTROL OF *Brochothrix thermosphacta* ON PORK

G. Gordon Greer, Bryan Dilts and Alenka Paquet

Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C & E Trail, Lacombe, Alberta, Canada T4L 1W1

Keywords: *Brochothrix thermosphacta*, myristoyl-L-methionine, pork

Background:

Long chain N-acyl derivatives of essential amino acids (Paquet, 1980) are a novel group of antimicrobials with reported antibotulinal activity in meat slurries (Paquet and Rayman, 1987). In commercial culture media, myristoyl derivatives of amino acids were the most active in efficacy trials using clostridia, *Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* (McKellar et al., 1992). Previous research was restricted to the susceptibility of Gram positive pathogens in commercial broths and there was a need to assess efficacy against spoilage bacteria in meat. *Brochothrix thermosphacta* can metabolize glucose and amino acids in raw, chilled meats to produce objectionable odours (Dainty and Mackey, 1992). Control of these bacteria could extend storage life in both aerobic environments and in vacuum packages with reduced levels of residual oxygen.

Objectives:

The objective of the current study was to compare the antibacterial efficacy of myristoyl-L-methionine (Myr-Met) in commercial broths, aqueous extracts of pork muscle and on pork lean tissue inoculated with *B. thermosphacta*.

Methods:

Myr-Met was prepared from L-methionine hydrochloride and the succinimidyl ester of myristic acid in aqueous acetone in the presence of sodium hydroxide (Paquet, 1980). The sodium salt was prepared from equivalent amounts of sodium hydroxide and Myr-Met. Myr-Met and free myristic acid were solublized in dimethyl formamide (McKellar et al., 1992) while methionine and the sodium salt of Myr-Met were dissolved in distilled water. APT broth (Difco Laboratories, Detroit, Michigan) was prepared according to the manufacturer's instructions. Aqueous extracts of pork *longissimus thoracis* (LT) muscle were prepared by homogenizing lean, ground LT in an equal volume of distilled water and filtration of the expressed juice. A pork slurry growth medium was prepared by homogenizing sterile, ground LT muscle in sterile distilled water (1:1, w/v). Sterile pork LT muscle discs (10 cm^2) were ascpetically excised from LT muscle of normal muscle quality (pH 5.6) by published procedures (Greer and Jones, 1991).

B. thermosphacta was a spoiled beef isolate observed to produce objectionable odours when inoculated onto meat surfaces (Greer and Dilts, 1998). The organism was grown in APT broth at 25°C to the mid log phase of growth and inocula were prepared by dilution in 0.1% (w/v) peptone water. Filter sterilized antibacterial substances and bacterial inocula were added directly to aqueous culture media to give the desired concentration and applied to meat tissue discs by immersion for 15 sec.

Inoculated media and meat discs were incubated aerobically at 4°C and bacterial numbers were determined by plate counts on tryptic soy agar plus 0.6% yeast extract (Difco Laboratories) and converted to log colony forming units/ml (CFU/ml).

Results and Discussion:

As reported by McKellar et al. (1992), the bactericidal activity was associated with the intact Myr-Met molecule and neither free methionine or myristic acid were active against *B. thermosphacta* in APT broth (Table 1). Myr-Met could reduce *B. thermosphacta* to below recoverable levels in APT broth within 2 d of incubation and this effect was concentration dependent (Table 2).

Although 500 μg/ml Myr-Me could eliminate *B. thermosphacta* in APT broth and aqueous extracts of pork muscle, activity was reduced in a pork slurry and it was unable to control bacteria at meat surfaces (Table 3). This stresses the danger in extrapolating the practical value of food preservatives from studies with broths.

It was necessary to synthesize the salt of Myr-Met (Na-Myr-Met) to permit solubility at concentrations required to produce antibacterial effects on pork (Table 4). Under these conditions, 5,000 and 20,000 μg/ml could produce a 2 to 3 log reduction in *B. thermosphacta* numbers after 4 d of pork storage at 4°C.

If a mechanism of enhancing activity in meat could be found, Myr-Met may provide an acceptable means of preservation. A toxiological evaluation by Health Canada (Arnold et al., 1998) showed that Myr-Met was readily metabolized and non-toxic to rats.

Conclusion:

Myr-Met is bactericidal to *B. thermosphacta* in aqueous culture media but loses activity in meat. The reason for inactivation in meat is unknown but must be resolved if this compound is to be recommended as a preservative.

References:

Arnold, D.L., McGuire, P.F., Miller, D., Malcolm, S., Hayward, S. and Paquet, A. 1998. The ability of the rat to metabolize myristoyl-methionine: An acylamino acid with potentially useful antibacterial properties. Food Chem. Toxicol. 36:771-779.

Dainty, R.H. and Mackey, B.M. 1992. The relationship between the phenotypic properties of bacteria from chill-stored meat and spoilage processes. J. Appl. Bacteriol. 73:103S-114S.

Greer, G.G. and Jones, S.D.M. 1991. Effects of lactic acid and vacuum packaging on beef processed in a research abattoir. Can. Inst. Food Sci. Technol. J. 24:161-168.

Greer, G.G. and Dilts, B.D. 1998. Bacteriophage control at meat spoilage by *Brochothrix thermosphacta*. 44th Proc. Int. Cong. Meat Sci. Technol. (Barcelona, Spain) pp. 324-325.

McKellar, R.C., Paquet, A. and Ma, C.Y. 1992. Antimicrobial activity of fatty N-acylamino acids against Gram-positive foodborne pathogens. Food Microbiol. 9:67-76.

Paquet, A. 1980. Preparation of some long-chain N-acyl derivatives of essential amino acids for nutritional studies. Can. J. Biochem. 58:573-576.

Paquet, A. and Rayman, K. 1987. Some N-acyl-D-amino acid derivatives having antibotulinal properties. Can. J. Microbiol. 33:557-582.

Table 1. Effect of Myr-Me and constituents on the aerobic growth of *B. thermosphacta* in APT broth at 4°C[1]

Time (d)	log CFU/cm^2			
	Control	Myristic acid (500 µg/ml)	Methionine (500 µg/ml)	Myr-Met (500 µg/ml)
0	3.88	4.03	4.25	3.04
2	5.20	4.27	5.75	1.00
7	9.37	8.41	9.61	1.00

[1]Data are means of 5 cultures.

Table 2. Effects of Myr-Met concentration on the aerobic growth of *B. thermosphacta* in APT broth at 4°C[1]

Time (d)	log CFU/cm^2			
	Myr-Met (µg/ml)			
	0	5	50	500
0	3.88	4.29	3.83	3.04
2	5.20	5.49	1.00	1.00
7	9.37	9.72	1.72	1.00

[1]Data are means of 5 cultures

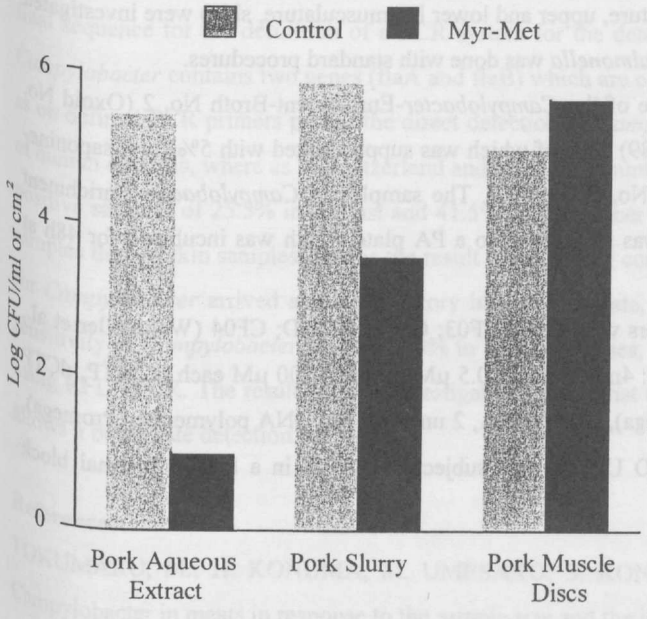

Figure 1. Inhibition of *B. thermosphacta* by Myr-Met. Bacteria inoculated media were incubated with 500 µg/ml of Myr-Met and incubated aerobically for 4d at 4°C. Data are means of 5 cultures or 5 muscle discs.

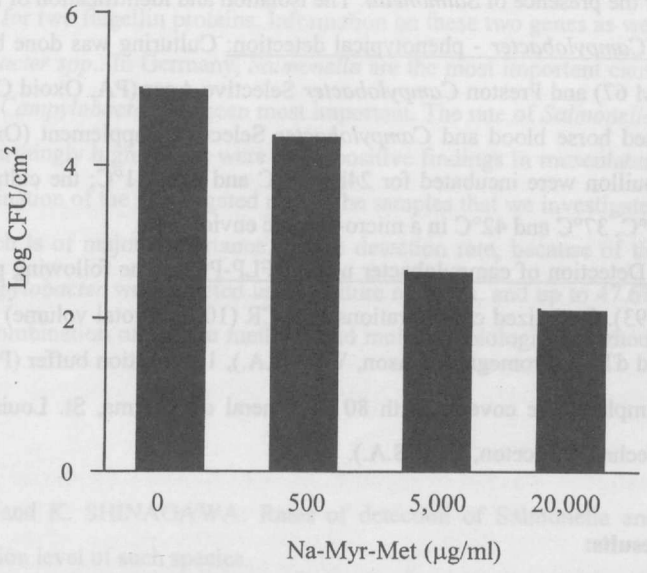

Figure 2. Inhibition of *B. thermosphacta* on pork muscle discs by the sodium salt of Myr-Met (Na-Myr-Met). Bacteria inoculated muscle was treated with Na-Myr-Met and incubated aerobically for 4d at 4°C. Data are means of 5 muscle discs.