ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA FROM MEAT ORIGIN AGAINST SELECTED INDICATOR MICROORGANISMS

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SUMMARY: The antimicrobial (bacteriocin) activity of selected lactobacilli and pedicocci previously isolated from Spanish dry fermented sausages has been evaluated against a number of selected indicator microorganisms, the reason for this screening being to identify suitable bacteriocin producers from which further biochemical, inmunological and molecular biology work could be performed. The antimicrobial activity of four lactobacilli and four pediococci of interest was evaluated not only against related microorganisms from the genera Lactobacillus, Pediococcus, Leuconostoc, Lactococcus, and Streptococcus, but also against spoilage and pathogenic representatives of the genera Bacillus, Clostridium, Propionibacterium, Listeria and Staphylococcus. None of the isolates was active against any of the Gram-negative bacteria tested, which included, among others, S. typhimurium and Y. enterocolitica. Supernatants from lactobacilli 148 are active against closely related bacteria and Gram-positive representatives of the genera Clostridium, Propionibacterium, Listeria and Staphylococcus, but not against B. cereus. The lactobacilli numbers 77, 86 and 156 displayed also an interesting range of antimicrobial activities. The four pediococci examined displayed, in general, a strong antimicrobial action against Listeria monocytogenes and Staphylococcus aureus.

INTRODUCTION: The lactic acid bacteria have the potential to inhibit the growth of pathogenic and spoilage bacteria and the possibility exists of using them to improve the hygienic quality and to extend the self-life of different meat and meat products (RACCACH et al., 1979; RODRIGUEZ et al., 1989; SCHILLINGER and LUCKE, 1989). Reduction of pH and removal of carbohydrates are the primary effects exerted by those bacteria (DAESCHEL, 1989), but they are also capable of producing other inhibitory substances such as hydrogen peroxide, diacetyl, bacteriocins and secondary metabolites that are antagonistic toward other microorganisms (KLAENHAMMER, 1988). Preservation is generally induced by organic acid formation, and the resulting pH decrease exhibits an antagonistic effect on microbial contaminants. However, since organic acids do not invariably destroy spoilage organisms and pathogens and a high level of acidity is not desirable in some products, the efficiency of organic acids as antimicrobial agents is relatively less significant in some cases (DABA et al., 1991). Accordingly to these reasons, a large effort is actually being done towards the isolation, identification and characterization of bacteriocinogenic lactic acid bacteria and the bacteriocins produced by them. Bacteriocinogenicity is the ability of bacteria to synthesize and release into their environment proteins which are toxic to other bacteria (DAESCHEL et al., 1990). Bacteriocins are interesting to the meat industry for its possible uses as food preservatives, once they have been adequately characterized. With increasing concern over chemical additives and preservatives, bacteriocinogenic bacteria or the bacteriocins produced by them could have an important role in food processing. We report in this communication the antimicrobial (bacteriocin) activity of selected lactobacilli and pediococci from meat origin against selected indicator microorganisms.

MATERIALS AND METHODS

Microorganisms, microbial growth and cell-free cultures: Four lactobacilli and four pediococci selected from a large number of isolates from Spanish dry fermented sausages and isolated during its fermentation, drying or from the final commercial product were considered. All isolates were grown at 32 °C until stationary phase on MRS broth (Oxoid). Corresponding cell-free solutions were obtained by centrifuging the cultures at 12,000 g for 10 min. This was followed by neutralization of the supernatants to pH 6.1 with 1N NaOH and filtration through a 0.22 µm pore size filters (Millipore). Aliquots of the cell-free culture supernatants were also lyophilised and afterwards resuspended in 4 mM phosphate buffer pH 7.0 to a concentration corresponding to twenty fold of the original concentration.

Measurement of the antimicrobial activity: An agar diffusion test was used; briefly, 20 ml of seeded solid agar media containing about 1×10^5 cfu/ml of the selected indicator strain was poured in a plastic petri dish. According to the indicator strain, the growth media used were either MRS, BHI (Brain Heart Infusion, Oxoid), RCM (Reinforced Clostridial Medium, Oxoid) or Glucose YE lemco medium (composition per liter of distilled water: peptone, 10 g; meat extract, 10 g; NaCl, 5 g; D-glucose, 5 g; yeast extract, 3 g; agar 15 g, with the pH ajusted to 7.0), the latter for growth of Propionibacterium strains. Seeded plates were maintained for 1h at 37 °C and holes of 6 mm diameter were performed in the agar. When 50 µl of supernatants from lactobacilli and pediococci strains were added to each well, plates were pre-incubated for 2h at 4 °C and further incubated under the conditions essential for growth of the target strains (aerobically,

anaerobically, 1-3 days, etc.). At the end of the incubation period the diameter of the inhibition zone (in mm) was measured.

RESULTS AND DISCUSSION

Four lactobacilli and four pediococci, selected from a large number of isolates from Spanish dry fermented sausages, were assayed for their antimicrobial (bacteriocin) activity against selected indicator microorganisms which included not only related microorganisms from the lactic acid bacteria group but also pathogenic and spoilage representatives of the genera Bacillus, Clostridium, Propionibacterium, Listeria and Staphylococcus. None of the isolates was active against any of the Gram-negative bacteria tested, which included, among others, S. typhimurium and Y. enterocolitica. The reason for this screening has been to identify suitable bacteriocin producers from which further biochemical, inmunological and molecular biological work could be performed, and complements efforts performed by our group (RODRIGUEZ et al.,1989; SOBRINO et al., 1991) and others (SCHILLINGER and LUCKE, 1989; LEWUS et al., 1991), in an effort to identify and select lactic acid bacteria from meat origin with bacteriocinogenic activity against foodborne bacterial pathogens.

Results obtained (Table I) indicates that supernatants from lactobacilli 148 are active against closely related bacteria and Gram-positive representatives of the genera Clostridium, Propionibacterium, Listeria and Staphylococcus. Lactobacilli 148 had been previously identified as L. sake 148 (SOBRINO et al., 1991). The lactobacilli numbers 77, 86 and 156 displayed also an interesting range of antimicrobial activities. The four pediococci examined displayed, in general, a strong antimicrobial action against Listeria monocytogenes and Staphylococcus aureus. The general objetives in searching for a desirable bacteriocin are that it be produced extracellularly from a food-safe microorganism and that it effectively antagonize undesirable bacteria which contaminate the foods (DABA et al., 1991).

The antimicrobial activity of and immunity to of lactobacilli 77 and 148 and pediococci 211 and 347, were evaluated against each other microorganism. Results (Table II) indicates that lactobacilli 148 inhibited growth of lactobacilli 77 and both pediococci, while being immune to its activity. These results indicate that lactobacilli 148 would inhibit growth of pediococci 211 and 347 in mixed cultures neglecting the usefulness of these mixed cultures and their use as protective cultures in meat substrates, unless molecular biology techniques could permit the expression of both activities in a single bacterium or the use of mixtures of purified bacteriocins. The results obtained also permit to genetically relate bacteriocin activity and immumity in a number of Bac$^-$ derivatives of the cited strains.

TABLE I. Antimicrobial (bacteriocin) activity of four lactobacilli and four pediococci from meat origin against selected indicator microorganisms[a]

		Lactobacilli Nº								Pediococci Nº							
		77		86		148		156		144		211		347		525	
Indicator species	strain nº	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Lactobacillus acidophilus	ATCC4356	-	-	-	-	7.4	10.8	-	-	-	-	-	-	-	-	-	-
Lactobacillus bulgaricus	ATCC11842	9.0	16.4	11.0	15.3	10.2	20.6	10.6	19.1	11.0	19.2	16.4	24.0	13.0	14.6	-	12.6
Lactobacillus casei	ATCC334	-	-	-	-	-	11.4	-	-	-	-	-	9.8	-	-	-	-
Lactobacillus curvatus	NCFB2739	-	11.6	-	-	10.0	16.4	-	-	-	-	12.2	19.6	13.6	20.4	15.0	20.2
Lactobacillus fermentum	ATCC9338	-	9.0	-	12.0	-	9.8	-	9.7	-	13.0	-	11.6	-	11.0	-	14.0
Lactobacillus helveticus	ATCC15009	-	-	-	-	-	10.2	-	-	-	-	-	-	-	-	-	-
Lactobacillus plantarum	NCDO1193	-	-	-	-	-	9.0	-	-	-	-	9.8	15.2	8.4	14.4	8.0	15.6
Lactobacillus reuteri	DSM20016	-	-	-	-	9.0	13.8	-	-	-	-	-	-	-	-	-	-
Lactobacillus sake	NCFB2714	15.4	20.6	-	-	15.2	21.0	9.6	17.0	10.8	16.0	29.2	34.0	31.0	38.6	31.0	33.6
Lactobacillus salivarius	NCFB2747	-	-	-	-	7.2	12.2	-	-	-	-	10.0	13.0	9.8	14.2	9.2	13.0
Pediococcus pentosaceus	FBB63	16.4	23.6	-	-	15.8	22.0	-	10.0	-	10.0	31.2	33.0	31.0	33.6	29.4	33.8
Pediococcus pentosaceus	PC1	-	12.6	-	-	9.6	17.8	-	-	-	13.5	-	-	-	-	-	-
Leuconostoc cremoris	DB1275	-	13.4	-	-	10.8	18.6	-	8.2	-	-	-	-	-	-	-	-
Lactococcus cremoris	CNRZ117	16.2	21.2	-	-	13.6	21.4	-	-	-	7.8	28.4	33.0	27.6	31.8	28.2	31.4
Enterococcus faecalis	EF	-	10.2	-	8.5	9.6	13.4	-	-	-	8.5	18.8	24.2	19.6	25.0	20.8	23.4
Staphylococcus carnosus	MC1	-	-	-	8.7	-	12.0	-	11.5	-	10.1	15.4	18.4	15.4	18.4	15.4	19.0
Listeria innocua	BL86/26	-	7.2	-	10.0	9.4	13.8	-	11.4	-	9.0	16.4	19.0	15.2	18.2	15.4	17.8

TABLE I.- (Continuation)

Indicator species	strain nº	Lactobacilli Nº								Pediococci Nº							
		77		86		148		156		144		211		347		525	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Clostridium sporogenes	C22/10	-	-	-	-	7.8	13.0	-	-	-	-	7.6	11.2	7.2	10.4	7.2	11.0
Clostridium tyrobutyricum	3,5CT	12.6	23.2	-	-	15.0	20.4	9.1	20.8	-	-	-	-	-	-	-	-
Clostridium tyrobutyricum	NCDO1754	14.8	24.2	-	-	17.8	24.0	9.2	17.2	-	11.6	-	-	-	-	-	-
Prop. acidipropionici	NCDO573	-	-	-	-	14.0	19.0	-	-	-	-	17.0	22.0	16.6	24.0	15.6	20.8
Propionibacterium sp	P4	12.0	23.6	-	-	9.6	17.4	-	10.0	-	-	13.0	20.0	13.4	20.6	13.0	20.2
Propionibacterium sp	P6	-	19.2	-	-	17.0	23.0	9.4	17.7	-	9.0	-	-	-	-	-	-
Clostridium perfringens	CECT376	-	-	-	-	8.4	13.0	8.2	12.5	-	-	-	-	-	-	-	-
Clostridium botulinum	CECT551	-	-	7.5	11.0	8.0	10.8	7.6	11.3	7.8	12.8	9.2	10.4	-	-	-	-
Listeria monocytogenes	NCTC7973	-	8.0	-	9.0	11.0	12.6	-	13.3	-	9.1	15.0	18.8	15.6	18.6	16.0	18.6
Listeria monocytogenes	LI5sv1/2	-	7.6	-	8.4	10.0	13.4	-	12.0	-	9.0	16.6	19.0	16.6	18.8	16.4	18.8
Listeria monocytogenes	NCTC5105	-	8.0	-	8.8	9.0	13.0	-	11.7	-	9.6	15.6	19.6	16.4	18.6	16.0	19.2
Listeria monocytogenes	LI1sv4	-	7.4	-	8.6	11.0	14.0	-	12.1	-	10.1	17.0	19.0	16.4	19.4	16.4	19.0
Listeria monocytogenes	ScottA	-	8.4	-	9.2	9.0	14.2	9.0	12.3	-	10.5	15.6	19.4	17.2	18.8	16.8	18.6
Staphylococcus aureus	FRI137	-	24.4	-	10.4	-	27.2	-	11.0	-	10.4	16.6	19.0	16.4	17.6	15.4	17.6
Staphylococcus aureus	FRI196E	-	-	-	9.6	-	14.0	-	10.8	-	10.2	-	-	-	-	-	-
Staphylococcus aureus	FRI349	-	8.0	-	8.7	-	13.0	-	10.5	-	9.5	15.2	19.6	16.4	18.6	15.4	19.4
Staphylococcus aureus	FRI472	-	-	-	9.3	-	16.4	-	11.1	-	9.6	-	-	-	-	-	-
Staphylococcus aureus	FRI361	-	-	-	9.0	-	11.4	-	10.4	-	9.9	16.6	21.2	15.4	17.6	15.6	21.0

[a] Diameter of inhibition zone in mm.
A. Supernatant
B. Supernatant 20-fold concentrated.

TABLE II. Antimicrobial activity of and immunity to of two lactobacilli and two pediococci from meat origin [a]

Indicator species (*)	Lactobacilli Nº				Pediococci Nº			
	77		148		211		347	
	A	B	A	B	A	B	A	B
Pediococcus sp	-	10.8	7.5	13.6	21.8	23.5	21.0	23.6
Lactobacillus Nº77	-	-	-	13.3	18.7	24.7	18.9	25.7
Lactobacillus Nº148								
Pediococcus Nº211	-	13.4	8.1	16.6	-	-	-	-
Pediococcus Nº347	-	13.7	8.6	15.9	-	-	-	-
Lactobacillus Nº77 (1n)	-	-	-	12.1	17.3	22.1	17.3	22.6
Lactobacillus Nº77 (2n)	-	-	-	12.7	16.8	23.4	16.8	23.7
Lactobacillus Nº77 (3b)	-	-	-	12.0	17.6	24.2	17.7	24.3
Lactobacillus Nº148 (1n)	-	10.5	-	11.3	-	-	-	-
Lactobacillus Nº148 (2n)	-	10.7	-	10.9	-	-	-	-
Lactobacillus Nº148 (3n)								
Pediococcus Nº211 (1b)	-	9.5	-	14.1	-	-	-	-
Pediococcus Nº211 (2b)	-	9.0	-	13.8	-	-	-	-
Pediococcus Nº211 (3b)	-	13.8	8.2	16.7	-	-	-	-
Pediococcus Nº211 (4b)	-	8.5	-	13.2	-	-	-	-
Pediococcus Nº347 (1n)	-	13.3	-	16.1	-	-	-	-
Pediococcus Nº347 (2n)	-	13.4	-	16.3	-	-	-	-
Pediococcus Nº347 (3n)	-	13.4	8.6	16.8	-	-	-	-
Pediococcus Nº347 (4n)	-	13.5	8.8	16.4	-	-	-	-
Pediococcus Nº347 (5n)	-	8.8	-	13.2	-	-	-	-
Pediococcus Nº347 (6b)	-	13.2	8.1	15.7	-	-	-	-
Pediococcus Nº347 (7b)	-	14.6	9.0	17.3	-	-	-	-

[a] Diameter of inhibition zone in mm
(*) Treated with Novobiocine (n) or Acriflavine (b)
A, B as in Table I

TABLE III. Antimicrobial activity of supernatant mixtures from lactobacilli and pediococci against selected indicator microorganisms[a]

Indicator species	strain Nº	Supernatant mixtures from											
		77+148		77+211		77+347		148+211		148+347		148+77+211+347	
		A	B	A	B	A	B	A	B	A	B	A	B
Enterococcus faecalis	EF	-	12.6	15.0	19.2	15.0	21.4	13.6	19.6	14.4	20.8	15.0	19.4
Staphylococcus carnosus	MC1	-	12.1	11.6	16.4	13.4	18.3	12.9	17.0	12.9	16.5	12.9	16.0
Listeria innocua	BL86/26	-	12.1	11.6	16.4	13.4	18.3	14.0	18.3	13.4	18.0	13.0	17.0
Clostridium sporogenes	C22/10	-	11.5	7.2	11.0	-	9.8	8.4	12.0	7.2	11.6	6.6	10.2
Clostridium tyrobutiricum	3,5CT	9.2	22.5	-	-	-	-	9.1	21.3	9.1	21.2	9.1	20.0
Clostridium tyrobutiricum	NCDO1754	10.2	21.0	-	-	-	-	9.3	19.0	9.6	17.6	8.5	15.0
Prop. acidipropionici	NCDO563	9.1	13.1	-	-	-	-	13.5	22.4	13.7	21.0	13.3	20.3
Propionibacterium sp.	P4	8.6	12.1	10.0	17.8	9.0	15.9	10.4	16.4	9.7	17.0	9.5	15.5
Propionibacterium sp.	P6	-	19.1	-	-	-	-	11.4	19.3	11.3	19.2	-	16.5
Listeria monocytogenes	NCTC7973	-	13.3	13.3	17.7	13.7	17.5	13.3	17.4	13.3	17.1	13.3	17.1
Listeria monocytogenes	Li5 sv 1/2	-	13.2	13.1	18.2	14.3	18.3	13.2	18.2	13.2	17.5	13.1	17.5
Staphylococcus aureus	FRI349	-	12.0	13.6	17.6	14.0	17.7	12.6	17.6	13.1	17.6	13.6	17.2
Staphylococcus aureus	FRI361	-	12.3	13.0	18.0	12.8	18.6	12.3	17.4	12.7	16.9	13.0	16.5

[a] Diameter of inhibition zone in mm
A, B as in Table I

The antimicrobial activity of supernatant mixtures from lactobacilli and pediococci of interest, have shwon that mixtures from lactobacilli 148 plus either pediococci 211 or 347 (Table III), are more effective than those of lactobacilli 77 plus lactobacilli 148 or lactobacilli 77 plus either pediococci, against a number of selected lactic acid bacteria. Once purified, the use of mixtures of bacteriocins as a natural means of preservation in a variety of foods, mostly those particularly susceptible to growth of psychrothrophic pathogens, may meet consumer demands for foods free of chemicals and preservatives and deserves further research.

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DETECTION OF SPOILAGE BACTERIA DURING BEEF PROCESSING - A COMPARISON OF THE CONDUCTANCE, VIABLE COUNT AND CONTAMINATION INDEX TECHNIQUES

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SUMMARY

The extent of the contamination of beef carcasses and meat cuts by different types of spoilage bacteria during slaughtering, chilling and cutting was monitored by three different analytical methods, each allowing estimates of the numbers of *Pseudomonas, Enterobacteriaceae,* and total aerobic bacteria to be made. The methods employed were those of (1) conventional viable count determination, (2) conductance monitoring and (3) a contamination index referring to spoilage bacteria present in small numbers prior to storage and calculated from viable count determinations made at fixed intervals during the storage of samples excised from carcasses. The conductance measures were found to correlate significantly with the corresponding viable count determinations. A bacteriological survey of a beef processing line indicated conductance monitoring to be capable of detecting small numbers of spoilage bacteria within virtually the same low range as the sensitive contamination index, and better than the viable count determinations did.

INTRODUCTION

The numbers and composition of spoilage microorganisms initially present on the meat surface influence the shelf-life of meat. *Pseudomonas* spp. are usually dominant on aerobically cold-stored meat (BLICKSTAD and MOLIN, 1983; DAINTY et al., 1983).

In addition, *Enterobacteriaceae* may reach high numbers during storage (BLICKSTAD et al., 1981). Total aerobic counts are frequently used for surveying bacteriological status of carcasses along the processing line in meat production (SNIJDERS, 1988; STOLLE, 1988). The use of viable count determinations on beef carcasses and meat during processing is not satisfactory due to the time required for analysis and for detecting bacteria which are present in very small numbers. A storage period for excised carcass surface samples at chill temperature was introduced in order to improve detection of small numbers of spoilage bacteria (GUSTAVSSON and BORCH, 1989). This approach has been shown to yield more accurate detection of low levels of spoilage bacteria, although a longer analysis time is required. The electrical detection of bacterial growth through conductance monitoring can be used for rapid and specific detection of bacteria (EASTER and GIBSON, 1989). In the present study three analytical methods (viable count determinations, conductance monitoring and contamination index) were compared in the estimates they provide of numbers of total aerobic bacteria, *Enterobacteriaceae* and *Pseudomonas* on beef carcasses and vacuum stored meat cuts.

MATERIALS AND METHODS

Experimental design: Five beef carcasses were followed along the processing line in one survey at a commercial abattoir in Sweden. Samples were taken (i) prior to rapid chilling, (ii) after 40 minutes in the rapid chiller at -8°C, (iii) after 48 hours of storage in the chiller at 1.5 to 4°C, (iv) after quarter-splitting and a further 24 hours of storage in chiller II at 4 to 5°C, and (v) after the cutting and deboning of the primal cuts. The cuts were then vacuum-packaged and stored at 4°C for up to five weeks. The date of slaughter was the same for all carcasses and the same carcasses were sampled at each of the separate stages of processing, except for the fact that, whereas five carcasses were sampled at stages (i), (ii) and (iii), four of them were sampled at stage (iv) and (v) and during the storage of meat cuts in vacuum packs.

Microbiological sampling: Surface samples were excised from the front section of the carcass (brisket, lateral forerib and foreleg) and from the stored vacuum-packaged meat cuts (lateral forerib). 12 meat pieces 2 cm in width (3.14 cm^2) and 1 cm in thickness were excised from the carcass at each sampling position along the processing line, using a sterilized cork borer. Four meat pieces were distributed and stored aerobically in stomacher bags. Three bags from each position and carcass were stored at 4°C and the separate bags were then analysed after 0, 7 and 14 days. The meat samples in each bag were homogenized with 25 ml of peptone-water (0.85 % NaCl, 0.1% peptone) in a stomacher for 35 seconds.

Viable count determination: Total aerobic counts were determined on Tryptone Glucose Extract agar (TGE, Oxoid, 25°C for 3 days), *Enterobacteriaceae* counts on Violet Red Bile agar (VRBG, Oxoid, 37°C for 1 day) and *Pseudomonas* counts on Pseudomonas C-F-C agar (Oxoid, 25°C for 2 days).

Calculation of the contamination index: The contamination index, reflecting the initial number of psychrotrophic spoilage bacteria present on the carcass was calculated as the sum of the viable counts of total aerobic bacteria, of *Enterobacteriaceae* and of *Pseudomonas*. The counts were obtained from analysis on fixed days (0, 7, 14 d) during the aerobic storage at 4°C of the samples excised from the carcasses.

Conductance monitoring: One ml of homogenate containing sample from the carcass surface (unstored) or from the vacuum stored meat cuts was used for inoculation. Total aerobic bacteria were monitored in 2 ml of SPYE broth (3.25% Special Peptone Yeast Extract, Malthus Instruments Ltd., Crawley, England), *Enterobacteriaceae* in 5 ml of ESM broth (1.45% Malthus Enterobacteriaceae Medium + 2.5% Skim Milk, Difco) and *Pseudomonas* in 1 ml of SPCFC (6.5% Special Peptone Yeast Extract supplemented with 50 μg/ml of cephaloridine, 50 μg/ml of fucidin and 50 μg/ml of cetrimide. All samples were run in a Malthus 2000 (Malthus Instruments Ltd.) incubator at 25°C for 50 hours. The detection time was indicated automatically by the software used (Malthus System Software, ver. H2.02.04). False detection times and false-positive detections were estimated graphically or were omitted.

RESULTS AND DISCUSSION

The selective conductance medium used for detection of *Pseudomonas* spp. was a modification of the Special Peptone Yeast Extract (Malthus Instruments Ltd., Crawley, England) with the supplement of cephaloridine, fucidin and cetrimide described by BANKS et al. (1987). The concentration of cephaloridine was decreased in the present study from 250 μg/ml to 50μg/ml since such a change improved the conductance detection of meat pseudomonads (unpublished data). The specific detection of *Pseudomonas* using conductance monitoring in SPCFC showed a significant correlation with viable counts on Pseudomonas C-F-C agar (Fig. 1a). The correlation was based on 29 samples from carcass surfaces and vacuum-packaged meat cuts. For three of the samples no colony-forming units were recovered on the C-F-C agar. Detection time for these samples was between 36 and 41 hours. This indicated that the conductance assay was able to detect *Pseudomonas* in very low numbers, in contrast to the viable count. The detection times varied from 5.5 to 48 hours, the viable counts varying between 0.6 and 6.6 log cfu/cm.

The selective conductance medium used for *Enterobacteriaceae* was a modification of the medium evaluated by COUSINS and MARLATT (1991). Through the addition of skim milk to their medium the detection of *Enterobacter agglomerans*, common on meat, was improved (ESM, unpublished data). The correlation between the detection times obtained in the ESM medium using conductance monitoring, and the *Enterobacteriaceae* counts on VRBG agar was significant (Fig. 1b). The correlation was based on 21 samples from carcasses and vacuum packaged meat cuts. The detection times for *Enterobacteriaceae* varied from 4.2 to 48 hours, the viable counts

varying between 0.3 and 6.9 log cfu/cm². The average detection time were shorter for *Enterobacteriaceae* than for *Pseudomonas*, due to the faster growth rate of *Enterobacteriaceae* in the selective media used for conductance monitoring.

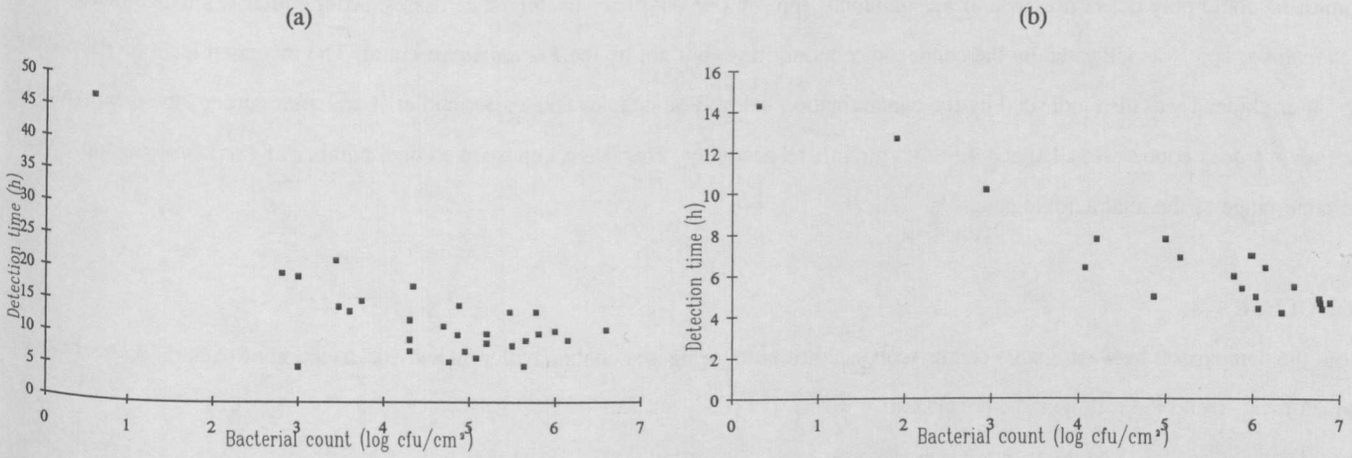

Fig. 1. Detection times in (a) SPCFC medium versus Pseudomonas counts on C-F-C agar; and (b) detection times in ESM medium versus *Enterobacteriaceae* counts on VRBG agar. Detection times were estimated with conductance monitoring.

The bacteriological survey of beef carcasses along the processing line, involving conductance, viable count and contamination index techniques, indicated increasing bacterial contamination (Table 1). The increase in the number of total aerobic bacteria after chiller II, and after cutting was pronounced, as all three methods indicated. In contrast, the increase occurring earlier on the processing line and after storage in chiller I was rather slight, as contamination index and the conductance monitoring results indicated. These two methods as opposed to viable count, were shown to be capable of detecting presence of small numbers of bacteria.

Table 1. Bacterial contamination of beef carcasses along the processing line estimated by viable count, detection time calculated from conductance monitoring and contamination index.

Position	Carcass no.	Viable count (log cfu/cm²)		Detection time (h)		Contamination index (log cfu/cm²)	
		Total bacteria	*Pseudomonas*	Total bacteria	*Pseudomonas*	Total bacteria	*Pseudomonas*
Before rapid chiller	1	0.9	<0	>50	>50	14.5	13.5
	2	3.2	<0	>50	>50	15.2	4.5
	3	1.1	<0	>50	>50	12.1	9.5
	4	2.5	<0	15.9	40.1	16.6	11.0
	5	1.8	<0	>50	>50	10.9	5.3
	mean	1.9	<0	>43	>48	13.9	8.8
After rapid chiller	1	2.1	<0	>50	>50	12.8	10.9
	2	2.4	<0	>50	>50	14.9	6.0
	3	2.3	<0	40.0	>50	13.5	6.6
	4	2.5	<0	20.6	>50	15.7	6.3
	5	2.2	<0	26.6	>50	14.8	7.1
	mean	2.3	<0	37.4	>50	14.3	7.4
After chiller I	1	1.8	<0	34.9	>50	17.2	10.9
	2	3.4	<0	33.3	35.7	20.6	14.3
	3	3.3	0.6	31.4	46.9	19.1	16.2
	4	3.6	<0	20.6	41.3	21.6	18.0
	5	0.4	<0	34.3	>50	14.9	6.0
	mean	2.5	<0.1	30.9	>44.8	18.7	13.1
After chiller II	1	3.5	2.8	22.0	20.0	27.2	25.6
	2	3.8	3.0	16.0	19.5	27.2	25.9
	3	6.9	6.6	11.0	11.2	33.3	33.3
	4	4.3	3.5	11.3	14.8	25.7	22.7
	mean	4.6	4.0	12.1	16.4	28.3	26.9
After cutting	1	5.8	5.8	11.1	14.0	31.0	31.0
	2	4.6	4.3	15.6	18.0	29.1	29.4
	3	7.2	>6	12.6	11.0	34.2	31.9
	4	3.9	3.4	18.5	22.0	28.8	28.0
	mean	5.4	>4.9	14.4	16.2	30.8	30.1

The number of *Pseudomonas* spp. as determined by viable counts was mainly below 1 cfu/cm², early on the processing line (before and after rapid chiller, and after chiller I; Table 1). In the first two processing positions (before and after rapid chiller) the conductance monitoring could only detect presence of *Pseudomonas* spp. on one out of ten examined carcasses. After chiller I, a contamination with *Pseudomonas* spp. was indicated by the conductance monitoring, but not by the *Pseudomonas* count. The increased level of *Pseudomonas* spp. after chiller I was also indicated by the contamination index. For samples taken after chiller II and after cutting, the mean values of the *Pseudomonas* counts were 4.0 and 4.9 log cfu/cm², respectively. This was an unexpected high number of *Pseudomonas* spp., and in the same range as the total aerobic count.

CONCLUSIONS

From the comparison between viable counts, conductance monitoring and contamination index, for detection of bacteria on beef carcasses and cut meat, could the following be concluded:

* conductance detection correlated with viable counts for estimation of *Pseudomonas* and *Enterobacteriaceae*

* conductance monitoring was as capable of detecting small numbers of total bacteria and *Pseudomonas* as the contamination index, and better than the viable count

* conductance monitoring was completed in less than 50 hours as opposed to days or weeks using the other methods in this study

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