PE6.04 Antimicrobial activity of Leuconostoc sp. LRCLe1 on beef 154.00

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Abstract— The antimicrobial activity of Leuconostoc sp. LRCLe1 on beef was studied and potential mechanisms for its antimicrobial activity were explored using culture media. No antimicrobial activity was evident in sterile culture supernatants although viable cells demonstrated activity. Activity was retained at 4ºC and under both aerobic and anaerobic incubation conditions. There was no evidence of the production of a proteinaceous substance. Sterile cores of fresh beef were coinoculated with LRCLe1 and Listeria monocytogenes or E. coli O157:H7. The presence of the pathogens did not affect the rapid growth of LRCLe1 in vacuum at 10ºC, but LRCLe1 inhibited the growth of both pathogens. When LRCLe1 was inoculated onto fresh commercial beef, it did not affect the sensory properties of the beef or the pH during 8 wk of storage in vacuum at 2 ºC. Numbers of Enterobacteriaceae, Pseudomonas spp. and Brochothrix thermosphacta were reduced by of 1.2, 1.3, and 1.0 log cfu/cm2 respectively. LRCLe1 may be valuable as part of a multifaceted antimicrobial strategy.

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Index Terms—microbial inhibition, antimicrobial activity, lactic acid bacteria, competitive inhibition

I. INTRODUCTION

THE Canadian Beef industry must produce a superior product if they are to compete domestically and in the global market place. The majority of raw beef product is shipped under vacuum packaged conditions and finding ways to prolong the storage life of meat products and eliminate the growth of pathogenic bacteria is vital to accessing world markets. Consumers are concerned about food-borne illnesses and the long-term effects of chemicals and preservatives added to food products. Finding natural methods of preservation should be viewed as positive steps in the consumer-driven marketplace.

Natural antimicrobials of microbial origin are of interest to the food industry. Meat microflora is comprised of a mixed population of both Gram positive and Gram negative bacteria, making the design and use of preservatives difficult. Refrigeration temperatures in vacuum packages select for the growth of psychrotrophic bacteria, predominantly lactic acid bacteria (LAB). Many species of LAB do not cause spoilage until they reach very high population numbers, and some can inhibit the growth of other bacteria including spoilage organisms. Lactic acid bacteria (LAB) are the predominant microflora in vacuum packaged meat. While LAB are implicated as the dominant spoilage causing organisms in these storage systems, they have also been demonstrated to show antimicrobial activity against Gram positive bacteria including Listeria monocytogenes (5, 8). Leuconostoc sp. LRCLe1 strain isolated from anoxically stored beef, inhibits Gram negative organisms such as Pseudomonas sp. and E. coli O157:H7 in vitro (12) and there is other evidence that LAB can inhibit Gram negative organisms. (3, 9).

This study was designed to test the antimicrobial activity of Leuconostoc sp. LRC-Le1 strain on sterile beef cores co-inoculated with Listeria monocytogenes and E. coli O157:H7, to evaluate the ability of Leuconostoc sp. LRCLe1 to compete with and inhibit the natural microflora on commercial beef and to measure its impact on sensory properties and pH of the meat.

II. MATERIALS AND METHODS

Deferred inhibition (10) analysis using viable cells, 4ºC incubation temperature, aerobic and anaerobic incubation conditions and with and without the introduction of pronase was used to determine whether the antimicrobial activity was related to a protein, the range of temperatures at which antimicrobial activity was retained and if the activity was displayed with and without oxygen. Direct spot on lawn analysis was used to detect

antimicrobial activity in the sterile supernatant A matrix assisted laser desorption/ionization – time of flight (MALDI-TOF) analysis was completed to determine the presence of a bacteriocin (Lynn McMullen, personal communication). The characterization of LRCLe1 was done parallel to characterization of Brochothrix campestris, a known producer of a bacteriocin. The growth of L.monocytogenes and E. coli O157:H7, alone and in the presence of equal numbers of LRCLe1 on sterile beef cores stored in vacuum packages at 10°C was examined.

Sterile cores of lean tissue were excised from beef loins. The cores were inoculated with approximately 2.5 log cfu/cm2 L. monocytogenes, E. coli O157:H7 or LRCLe1 for control samples. Treated samples were inoculated with 2.5 log cfu/cm2 L. monocytogenes and LRCLe1 or E. coli O157:H7 and LRCLe1.

Cores were packaged in vacuum, stored for 6 wk at 10ºC and at weekly intervals the numbers of the different bacteria were determined. The pH was measured through the course of storage using an Oktron Digital pH meter (Model Wo-0060500-000, Anachemia Scientific, Calgary, AB, Canada). Vacuum packaged beef loins were purchased from a commercial supplier.

The loins were cut into 2 kg pieces and treated by submerging the loin into a bath containing 4 log cfu/ml of LRCLe1 suspended in sterile water. (treated) or sterile water (control).

The samples were moved manually during 5 min of treatment time to assure an even exposure to the treatment. The loins were vacuum packaged and incubated at 2°C. Every 2 wk for 8 wk a loin from each treatment was removed. The pH was measured. Sensory evaluation consisting of the assessment of muscle colour (8-point scale: 1 = white; 8 = extremely dark red), surface discolouration (7-point scale: 1 = no surface discolouration; 7 = complete discolouration), retail appearance (7-point scale: 1 = extremely undesirable; 7 = extremely desirable), off-odour intensity (5-point scale: 1 = no off-odour; 5 = prevalent off-odour), and odour acceptability (5-point scale: 1 = acceptable; 5 = unacceptable) by an experienced, trained, 5-member sensory panel (4) was completed.

Also, 4 -10 cm2 cores were aseptically excised from each loin. Cores for each sample were combined and homogenized for 2 min in 10 ml of 0.1% peptone diluent using a Colworth Stomacher (Baxter Diagnostics Corp., Canlab Division, Edmonton, AB, Canada). Ten-fold dilutions were prepared and aliquots were plated. Selective media described by Baird et al. (2) were used to enumerate Pseudomonas spp., Brochothrix thermosphacta, LAB and Enterobacteriaceae and standard microbiological methods were used to enumerate psychrotrophs and mesophiles. The population of LAB on MRS plates was screened using the random amplification of polymorphic DNA (RAPD)(11) and the 239 primer (7) to determine whether close relatives of LRCLe1 were present in the natural population.

III. RESULTS AND DISCUSSION

The antimicrobial characteristics of LRCLe1 compared to those of B. campestris are presented in Table 1. LRCLe1 viable cells were inhibitory at refrigeration temperatures, when incubated under both aerobic and anaerobic conditions and to Gram –ve bacteria.

There was no antimicrobial activity in the sterile culture supernatant. Figure 1a shows the results of the spot on lawn assay using sterile supernatant and Figure 1b shows the lack of effect of pronase when included in a deferred inhibition assay. Although the mechanism of inhibition of LRCLe1 remains unclear, it retains its antimicrobial activity under normal refrigerated, anoxic meat storage conditions. As shown previously and confirmed in this study, its antimicrobial activity is not limited to Gram positive bacteria (12).

Results of antimicrobial activity in culture media cannot be translated to the meat environment with results in some cases being quite different. A previous study indicated that when LRCLe1 was present in high numbers, growth of enteric bacteria was inhibited (12).

The ability of LRCLe1, L. monocyotogenes and E. coli O157:H7 to grow at selected refrigeration temperatures showed that, although LRCLe1 could grow to maximum numbers at 4ºC, 7ºC and 10ºC (data not shown), L. monoctyogenes and E. coli O157:H7 only grew well at 10ºC which was the temperature selected for further trials. Growth on beef cores during 6 wk of vacuum packaged storage

at 10ºC of both E. coli O157:H7 and L. monocytogenes was inhibited when each organism was coinoculated with LRCLe1 (Fig. 2). Inoculation levels were adjusted to approximately 2.5 log cfu/cm2 to simulate the relatively low numbers of bacteria on fresh meat.

The growth of LRCLe1 was not affected by the presence of E. coli O157:H7 or L. monocytogenes (Fig. 2) and LRCLe1 reached maximum numbers (8 log cfu/cm2) after only 1 wk of storage. The pH of the cores did not change significantly when inoculated with LRCLe1 as a result of time and the mean ranged from 5.3 to 5.5 indicating that production of lactic and/or acetic acid (6) is unlikely to be the reason for inhibition.

LRCLe1 was isolated as the dominant bacterium on stored beef (12). Growth on beef cores was rapid. Requirements for appropriate inhibitory strains of lactic acid bacteria for use in ready-to-eat meats suggested by Amézquita and Brashears (1) are that they survive during storage at refrigeration temperatures, but not grow if they will spoil the product, and do not alter the sensory properties. When LRCLe1 was inoculated onto naturally-contaminated beef and samples were stored in vacuum at 2ºC for 8 wk, it had no effect on the pH of the meat (range 5.2 – 5.7), nor did it significantly affect the sensory properties of the meat. In Figure 3, the evaluation of retail appearance and odour acceptability are shown.

The retail appearance remained acceptable for 6 wk of the storage time and odour deteriorated to an unacceptable level between 4 (untreated) and 5 (treated) wk of storage. In preparation for evaluating the ability of LRCLe1 to reduce bacterial numbers on naturally-contaminated beef loins, RAPD analysis was done on LAB isolated from commercial beef.

Also, a selection of LAB recovered during storage of vacuum packaged beef was analyzed using RAPD. LRCLe1 has not been found in any of the samples analyzed (data not shown). Inoculation of loins with LRCLe1 resulted in decreases in the numbers of Enterobacteriaceae, Pseudomonas spp. and Brochothrix thermosphacta of 1.2, 1.3, and 1.0 log cfu/cm2 respectively after 8 wk of storage (Fig. 4).

Generally changes in bacterial numbers of 1 log cfu/cm2 or g are considered to be of practical significance. Reduced growth of bacteria with spoilage potential, such as Enterobacteriaceae, Pseudomonas spp. and B. thermosphacta, could be of benefit to the meat industry.

IV. CONCLUSION

The antimicrobial properties of LRCLe1, a Leuconostoc sp. isolated from vacuum packaged beef, that quickly grows to high numbers on meat at low temperatures were described. The organism does not spoil fresh beef stored in vacuum at refrigeration temperatures. It reduced numbers of spoilage bacteria by approximately 1 log cfu/cm2 after 8 wk of storage in vacuum at refrigeration temperature.

Alone, it may not useful as an antimicrobial, but it may form the basis of a hurdle strategy in the design of combinations of natural antimicrobials. Further research would be required to fully explain the mechanism of inhibition.

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