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Vacuum-Packed Beef Bacteria: Extrinsic and Intrinsic Factors that Determine Microbial Communities

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Abstract

Australian export-grade vacuum-packed (VP) beef primals have notably long shelf-life. While this is an envious position in international commerce, the specific factors that control this benefit are not well understood. Previous MLA projects measured changes in sensory and microbiological properties of VP striploins and cube rolls from six Australian export abattoirs, and found substantial differences in microbiological profiles. This led to a second phase of research that showed there were no unique bacterial species among the abattoirs to explain differences in primal shelf-life, indicating that the cause was more likely due to properties of specific bacterial strains. Results from the present study support this idea, showing that strains of bacteria on VP primals from abattoirs with low bacterial growth were more sensitive to pH, lactic acid and to low concentrations of glucose. More importantly, these abattoirs have a higher proportion of strains that produce inhibitory compounds against other bacteria, with the greatest effects against bacteria of the same species. As a result, these interactions may limit the overall growth of the bacterial community, resulting in longer shelf-life and a higher quality.

Executive summary

The shelf-life of high quality Australian vacuum-packed (VP) primals can exceed 16 weeks when product is stored at -0.5°C. While this is an enviable position for industry in global markets, at present, factors responsible for this extended shelf-life are not well defined. As a result, if product shelf-life falls below expectations, the specific quality control measures needed to correct this problem are not known.

In two previous MLA projects (Predictive Models for Spoilage in Vacuum Packed Primals [A.MFS.0147] and Microbial Communities in Stored Vacuum Packed Primals [A.MFS.0194], we systematically approached this problem. In the first study, collaborating with CSIRO, we modelled the growth of TVC and LAB bacteria in vacuum-packed (VP) beef primals and then compared model predictions with growth profiles for VP striploins and cube rolls produced at six Australian abattoirs. We found that while the model performed well for some abattoirs, in some cases it markedly over-predicted bacterial growth when bacterial numbers only increased 1-2 log CFU/cm^2 over 16-30 weeks of storage at -0.5°C.

In the second study, we tested the hypothesis that increases in TVC and LAB on VP primals vary among abattoirs due to different types and levels of bacteria that dominate during refrigerated storage. Using culture-independent methods consisting of Terminal Restriction Length Polymorphism (TRFLP) and clone library, we found that relative proportions of particular species did vary among the abattoirs. However, the inter-abattoir variation in bacterial growth was not associated with unique bacterial species.

As a result, our attention focused on understanding the responses of specific bacterial strains to intrinsic and extrinsic factors of meat. This was done by testing a large collection of bacteria for sensitivity to pH, glucose, organic acids and low temperature, as well as the ability to inhibit other bacteria. The results showed that bacterial strains, on primals from abattoirs with uniquely lower bacterial numbers, were more sensitive to lactic acid and to low concentrations of glucose. More importantly, a higher proportion of these isolates produced inhibitory compounds against other bacteria, with the greatest effect on bacteria of the same species. As a result, these interactions may be important in limiting the overall growth of the bacterial community, resulting in long shelf-life and a higher product quality.

In summary, these studies show that responses of specific bacterial strains to intrinsic properties of VP beef likely define the dominant microbial community, producing a less diverse bacterial flora dominated by LAB. However, bacterial interactions in the early phases of storage may be a critical step in suppressing Enterobacteriacae and *Pseudomonas* species that would otherwise dominate due to greater resistance to low pH and lactic acid, compared to LAB.

This research benefits the meat industry by providing a more thorough understanding of microbial communities associated with extended shelf-life of VP primals, and potential strategies to maintain these desirable bacteria. Additional studies are needed to validate these findings under controlled industry conditions.

Contents

1 Background

Vacuum-packaged Australian export primals are recognised for long shelf-life. Exporters observe that shelf-life may be as long as 75-100 days when stored in the range of -1 to 3ºC. However, the basis for this effect is not adequately understood. Consequently, it is not possible to properly design controls that are based on known mechanisms that produce a desired level of product quality and shelf-life.

It is well known that the shelf-life of fresh beef is affected by intrinsic and extrinsic factors, mainly temperature, pH, and packaging atmosphere that influence the viability, physiology and interactions among bacterial species. The effects of some of these factors were investigated in two earlier projects: Predictive Models for Spoilage in Vacuum Packed Primals (A.MFS.0147) and Microbial Communities in Stored Vacuum Packed Primals (A.MFS.0194). In the former project Total Viable Count (TVC) and Lactic Acid Bacteria (LAB) viability were modelled for vacuum-packed (VP) primals and validated against commercial products from six abattoirs.

Marked differences in growth profiles were observed among the abattoirs, and between striploins and cube rolls. These observations led to further research to determine if such differences were associated with changes in profiles of microbial communities on primals. Microbial communities were different among the six abattoirs, and between striploin and cube roll, by tRFLP analysis. Clone libraries showed, using a subset of abattoirs representing high and low TVC and LAB growth rates, that bacterial species differed among the abattoirs during 30 weeks of storage. However, there was no association between a specific profile of bacterial species and the level of growth of TVC and LAB on VP primals.

As a result, the present research tested the hypothesis that primals possessing lower bacterial growth results from differences in how individual bacterial strains respond to intrinsic and extrinsic properties of meat.

1 Project objectives

The overall objectives of the project were to:

- measure responses of bacterial isolates from six export abattoirs to the effects of pH, glucose, organic acids, temperature, and to determine if isolates produced growth-inhibitory compounds and,

- determine which of the properties described above are associated with lower levels of bacteria growth over product storage.

2 Methodology

2.1 Speciation of bacterial isolates

Isolates. The isolates used in this study were collected from the vacuum-packaged beef primals tested by CSIRO and described in MLA project “Shelf-life of Chilled Vacuum Packed Beef” (A.MFS.0166). Primals were sampled at 1, 8, 12, 16, 20, 24, 26, 28 and 30 weeks, and CSIRO shipped the frozen primal rinsates to the University of Tasmania. The isolation of bacteria from rinsates at selected time intervals is described in the project “Microbial Communities in Stored Vacuum Packed Primals” (A.MFS.0194).

From this collection of bacteria, a subset of 180 isolates was selected, representing the six abattoirs, three time intervals (1, 8 and 30 weeks), one replicate primal per time interval and 10 isolates per sample. For experimentation, isolates were transferred from -80°C storage, streaked on Tryptone Soya agar (TSA; Tryptone Soya broth, Oxoid Ltd, plus 15g/l agar (Gelita Australia Pty Ltd)) and incubated at 25°C for 1-4 days.

PCR. The 16S rRNA gene was amplified using the primers 10F (5'-GAGTTTG-ATCCTGGCTCAG-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3'). Amplicons were generated using a MJ Research PTC-200 peltier thermal cycler and the following program: 1 cycle of 10 minutes at 95°C; 35 cycles of 1 minute at 94°C, 1 minute at 55°C, 1 minute at 72°C; and a final extension step of 7 minutes at 72°C. The PCR product was checked on 1.5% agarose gel with GelRed, run at 100V for 30 minutes. The gel was visualised and photographed with BioRad Gel-Doc system and QuantityOne™ program. The samples were sent to Macrogen (Seoul, Korea) for purification and sequencing.

Sequence analysis**.** Raw sequence files were imported into BIOEDIT v. 7.0.5.3 where chromatograms were analysed for quality. The sequences were compared against others in the Genbank database using the BLAST function (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi). The closest matches for each clone were used to determine probable identities.

2.2 Effect of pH

An initial screening of 10 isolates, five from abattoir A week-30 and five from abattoir C week-30 (A30a, A30b, A30c, A30f, A30i, C30a, C30b, C30d, C30f and C30h), was conducted to establish a range of pH to test the full set of 180 isolates. Isolates were streaked on TSA and incubated at 25°C for 24-48 hours. Late stationary phase cultures were grown by inoculating Brain Heart Infusion broth (BHI; Amyl Media Ltd) (pH 7.2 ± 0.2) with 1-2 colonies and incubating at 37°C for 18 hours. Cultures were adjusted to approximately $1x10^8$ cfu/ml by measuring optical density (OD) at 540 nm (Spectrostar Nano, BMG Labtech, GmbH Germany) and diluting with BHI until an OD of 0.15-0.25 was obtained. Cultures were then serially diluted in 0.1% peptone (Bacteriological Peptone, Oxoid Ltd) to obtain 1 x 10^5 CFU/ml culture. Modified BHI (mBHI) was made by the addition of 17 mM tri sodium citrate (Amresco) (Grau, 1980) and adjusting the pH to 4.0, 4.5, 5.0, 5.5 or 6.0 using hydrochloric acid (Emsure, Merck KGaA) or 10% sodium hydroxide (Sigma-Aldrich Co. LLC). pH was measured using an Orion pH meter (model 250A, Orion Research inc, Boston MA, USA) Four and a half millilitres of mBHI were inoculated at $1x10^4$ cfu/ml, incubated at 25°C for 12 days, and the OD measured at 540 nm.

Following this initial screening, the 10 isolates were again screened in the Bioscreen C (Growth Curves Ab Ltd, Finland). Isolates were streaked on TSA and incubated at 25°C for 24-48 hours. Stationary phase cultures were produced by inoculating 1.25 ml BHI broth (pH 7.2 ± 0.2) with one or two colonies and incubating at 37°C for 18 hours. The OD of the culture was measured at 540 nm and adjusted to 0.15-0.25 to obtain 1 x 10^8 CFU/ml. Next, 10-fold serial dilutions were prepared in 0.1% peptone to produce an inoculum of 1 x 10^5 CFU/ml.

In the Bioscreen C plate, 315 µl of mBHI (pH 4.0, 4.5, 5.0, 5.5 and 6.0) was inoculated with 35 µl of diluted isolates to produce 1 x 10^4 CFU/ml. Isolates were measured in triplicate. Bioscreen C plates were then incubated in the instrument under the following conditions: 25°C for 4 days with medium shaking amplitude, normal speed shaking for 5 seconds at 10 second intervals. Readings were taken at

30 minute intervals. For positive controls, isolates were also grown in BHI pH 7.2±0.2 to verify growth under optimal pH. This screening showed that differential isolate growth patterns could be achieved after 24 hours, therefore subsequent Bioscreen C runs were performed over 24 hours.

Change in the pH change of culture media

Twelve isolates (A30a, A30c, A30f, A30h, A30g, A30j, C30b, C30d, C30f, C30h, C30i and C30j) were screened in 4.5 ml mBHI (pH 4.5, 5.0, 5.5 and 7.2), incubated for 48 hours and the pH of the medium measured. Following these initial screening studies, the full set of 180 isolates was tested at pH 4.5, 5.0 and 5.5. All isolates were screened as described previously in the Bioscreen C for 24 hours.

2.3 Effect of lactic acid

The lactic acid concentration in ground beef is approximately 72-94 mM (Nassos *et al*, 1983). An initial screening of selected isolates was conducted to determine the range of lactic acid to use for the full study. Lactic acid concentrations of 0, 50, 100 and 200 mM, at each pH used in the previous study (pH 4.5, 5.0 and 5.5), were prepared. Modified BHI was made with the addition of 17 mM tri-sodium citrate, lactic acid (Sigma-Aldrich Co. LLC) and the pH adjusted using hydrochloric acid or sodium hydroxide. The medium was sterilised at 121°C for 20 minutes. Twelve isolates were chosen for an initial screening representative of the range of growth responses observed in the pH study (isolates A0f, A30f, A30g, A30h, B8b, B8c, B30b, C30b, C30e, C30f, D0a and D0i). The isolates were screened in the Bioscreen C for 24 hours as previously described. Following this screening, growth profiles of the 180 isolates were determined at 50, 100 and 200 mM lactic acid, pH 5.5, for 24 hours at 25°C in the Bioscreen C.

2.4 Effect of acetic acid

An initial screening of selected isolates was done using acetic acid concentrations of 0.025% v/v (4.25 mM), 0.05% (8.5 mM), 0.1%(17 mM), 0.25% (42.5 mM), 0.5% (85 mM) and 1% (170 mM), at each pH used previously (pH 4.5, 5.0 and 5.5). Modified BHI was made with 17 mM tri-sodium citrate, acetic acid (Spectrum Chemical MFG Corp.), at the previously stated concentrations, and the pH adjusted using hydrochloric acid or sodium hydroxide. The medium was sterilised at 121°C for 20 minutes. Eleven isolates were chosen for an initial screening, using the range of growth responses observed in the pH study (isolates A30f, A30g, A30h, B8b, B8c, B30b, C30b, C30e, C30f, D0a and D0i). The growth profiles were measured in the Bioscreen C at 25°C for 24 hours. Following this screening, all 180 isolates were tested at 0.025, 0.05 and 0.1% acetic acid, pH 5.5 for 24 h at 25°C in the Bioscreen C.

2.5 Effect of glucose

Minimal Broth Davis without dextrose (BD Difco, Australia) was made by adding 10.6 grams dehydrated minimal broth media to 900 ml of distilled water. After autoclaving and cooling the medium, a filter-sterilised (0.2 µm, FP POINT 2-S, Whatman GmbH, Germany) glucose (Sigma-Aldrich Co. LLC) solution was added to the media to give final concentrations of 500 mM (9%), 200 mM (3.6%), 100 mM (1.8%), 75 mM (1.35%), 50 mM (0.9%), 25 mM (0.45%), 10 mM (0.18%), 5 mM (0.09%), 2.5 mM (0.045%), 1.0 mM (0.018%), 0.5 mM (0.009%) or 0.1 mM (0.0018%).

2.6 Effect of low temperature

Bioscreen plates were set-up as previously described, using BHI pH 7.2 ± 0.2. Due to the extended time for experimentation, plates were placed in plastic bags to prevent dehydration and then placed in a refrigerated incubator (Binder KB 115 or KB 240 incubator, Binder GmbH, Germany). Plates were incubated at -2.5, -1 and 1ºC, and removed just prior to reading the OD in the Bioscreen C. The Bioscreen C was set at 15°C (the lowest temperature-setting at room temperature), the plate shaker increased to "high" setting and the length of shaking increased to 20 seconds to resuspend bacterial cells. The OD readings were measured at 3-minute intervals until a stable OD value was obtained. Typically, plates were in the Bioscreen C for 6-18 minutes. On occasion, humid room conditions produced condensation on the exterior of the Bioscreen plates. In these circumstances, plates were placed in a laminar flow cabinet for several minutes prior to measuring OD to remove condensation. The sample temperature profile during incubation and OD measurements was measured by testing a separate uninoculated plate with a thermocouple. In addition, a duplicate set of plates was incubated without removal from the incubator for five weeks and the OD measured in the Bioscreen C.

2.7 Bacteria-bacteria Inhibition

Isolates were taken from the -80°C freezer, streaked on TSA and grown for 24 hours at 25°C. Overnight cultures were prepared by inoculating 1 ml of BHI broth with a single colony for each isolate and incubating broth for 24 hours at 25°C. Next, 10 µl of each overnight culture was spotted on TSA plates that had been pre-streaked with 100 µl of an overnight culture of the target strain (lawn), incubated at 25°C and examined after 24 hours for zones of inhibition (Singh and Prakash, 2009). Isolate-isolate interactions were tested only among isolates from a single abattoir (10 isolates from each of three time intervals; total 30 isolates per aattoir).

Quantifying inhibition

The strength of inhibition was quantified using the Bioscreen C. Isolates were grown on TSA as previously described. Test cultures were produced by inoculating one colony in approximately five millilitres of BHI broth and incubating for 18 hours at 25°C with shaking (200 rpm). Target isolates were diluted in BHI, if necessary, to obtain an OD of 0.15 to 0.25 at 540 nm. Cultures were serially diluted in 0.1% peptone to1 x 10^4 CFU/ml.

Effector (inhibiting) strains were diluted to obtain an OD between 0.6 and 0.8 at 540 nm. Some effector strains (in particular *Bacillus spp*.) did not reach the desired OD after 18 hours; in some cases the OD decreased if the cultures were incubated for longer periods. The OD of these strains was measured after 18 hours of incubation and the culture used at that level, regardless. The cultures were then centrifuged (Eppendorf microcentrifuge 5417R) at 1000 x *g* at 4°C for 5 minutes. The supernatant was removed and filtered through a 0.2 µm syringe filter. Doubling dilutions of the cell-free supernatant were performed in a Bioscreen C plate, up to 1/128, resulting in 100 µl of supernatant in the first eight wells of each column in the plate. The supernatant was then inoculated with 100 µl of the target culture (1 x 10^4 cfu/ml). In addition, 100 µl of the target culture was added to 100 µl BHI for the negative control. All reactions were performed in duplicate and repeated twice. Plates were incubated in the Bioscreen C at 25ºC as described previously, for at least 18 hours.

3 Results and Discussion

3.1 Speciation of bacterial isolates

The predominant isolates on primals from each abattoir were speciated by 16s rDNA sequencing. The time intervals and meat types were selected because they showed significantly different microbial communities by tRFLP and clone library as reported in "Microbial Communities in Stored Vacuum Packed Primals" (A.MFS.0194).

Abattoir A

At week-30, 70% of isolates were *Carnobacterium divergens*, increasing from 20-30% at week-1 and week-8 (Fig. 1). Four genera (*Bacillus*, *Hafnia*, *Brochothrix*, *Carnobacterium*) were present at week-1 and -8 (*Yersinia*, *Hafnia*, *Brochothrix*, *Carnobacterium*).

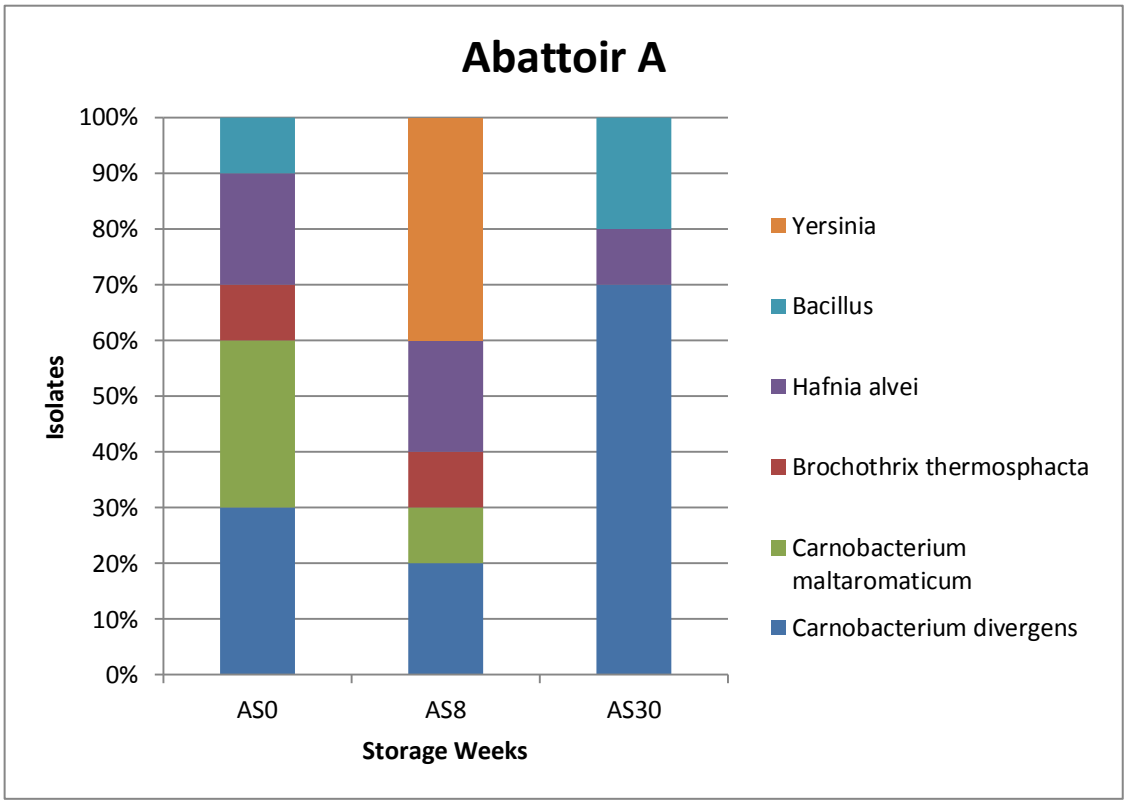

Figure 1. Abattoir A isolates from 1, 8 and 30 weeks storage.

Abattoir B

In contrast to abattoir A, *Carnobacterium* represented 30-50% of isolates at 1 and 8 weeks, but only 10% of isolates at 30 weeks (Fig.2). *Serratia* and *Pseudomonas* represented 50 and 20% of isolates at week-1, respectively. At week-8, *Rahnella*, *Pseudomonas*, and *Yersinia* each constituted 10% of isolates, with *Hafnia alvei* at 20%. At week-30, 10, 20 and 60% of isolates were *Leuconstoc*, *Bacillus* and *Serratia spp.*, respectively.

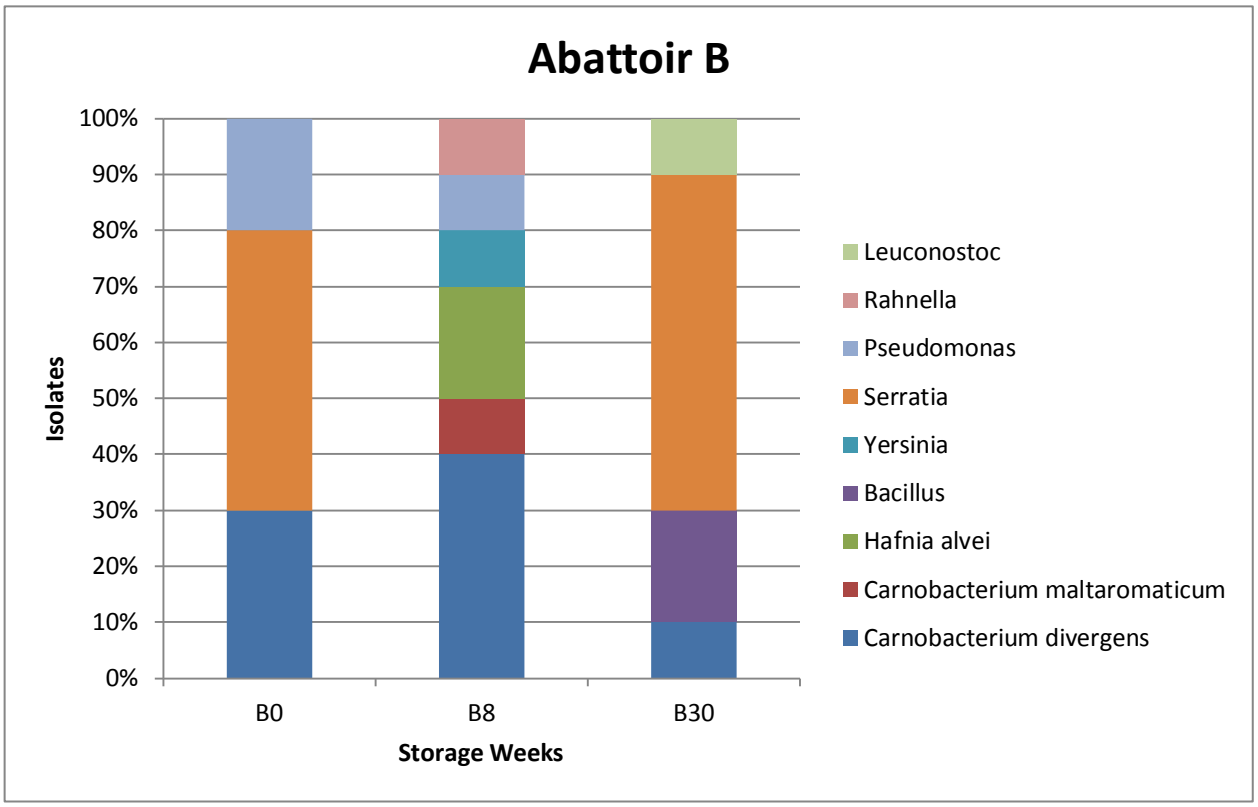

Figure 2. Abattoir B isolates at 1, 8 and 30 weeks.

Abattoir C

Carnobacterium species represented 10, 90 and 50% of the isolates at week-1, -8 and -30. (Fig. 3). LAB (*Carnobacterium* and *Leuconostoc)* represented 80% of isolates at week-30.

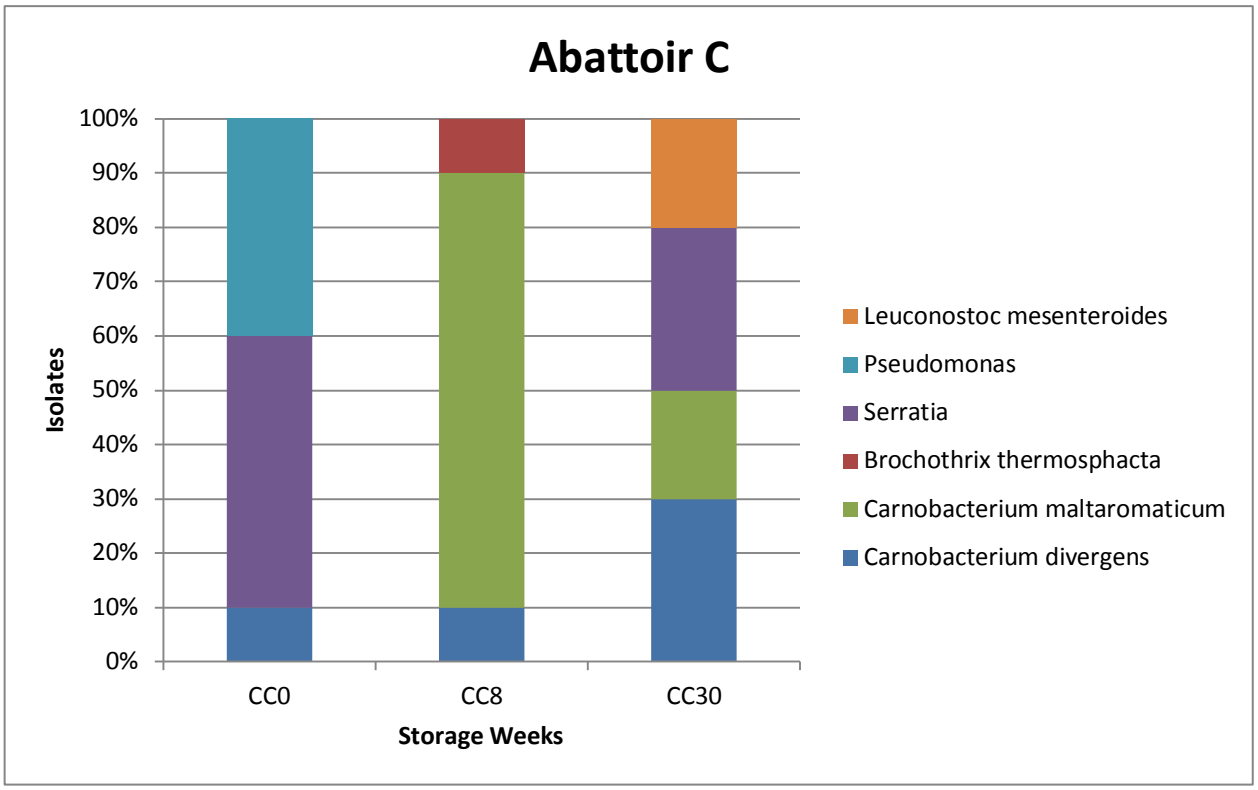

Figure 3. Abattoir C isolates at 1, 8 and 30 weeks.

Abattoir D

Abattoir D was unique in that 100% of the isolates at week-30 were *C. divergens* (Fig. 4). There was a progressive increase in *Carnobacterium* spp. from 20% at week-1, to 40% at week-8 and 100% at week-30. The proportion of *Pseudomonas* declined from 40 to 30 to 0%, from one to 30 weeks.

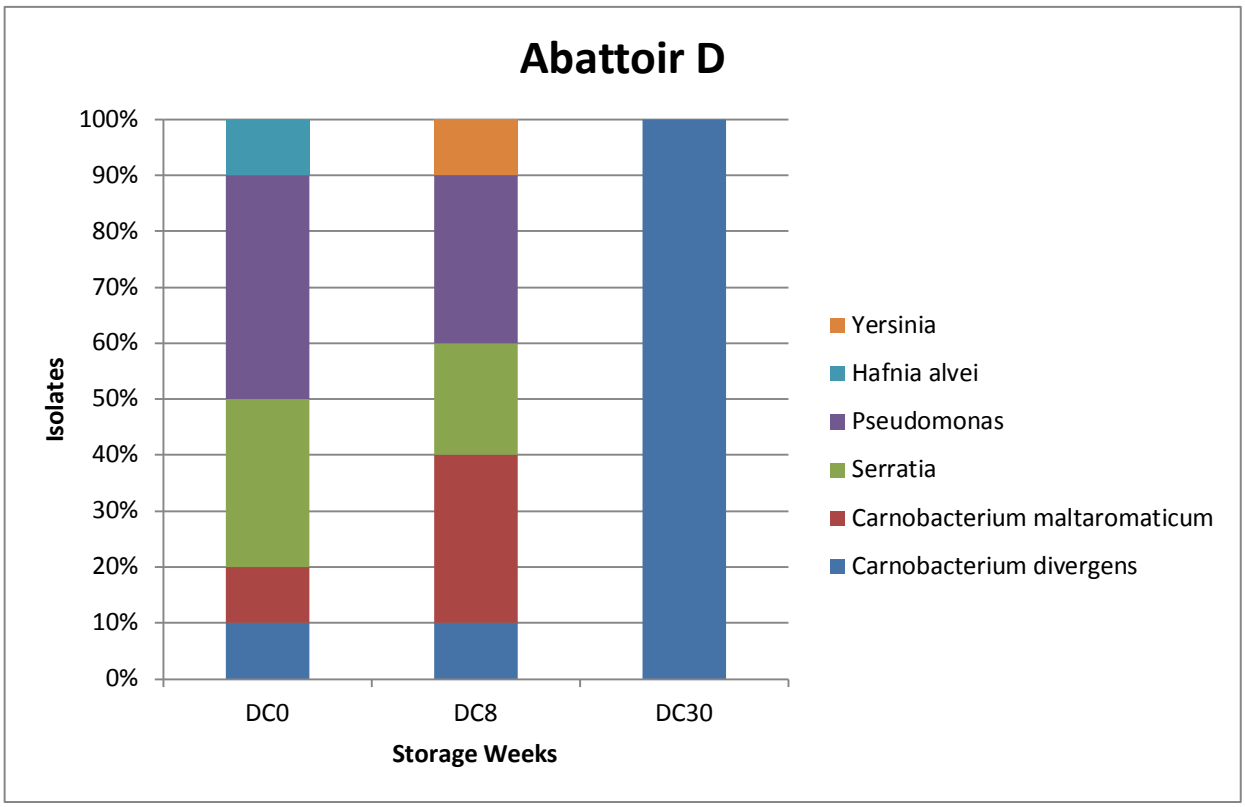

Figure 4. Abattoir D isolates at 1, 8 and 30 weeks.

Abattoir E

Carnobacterium maltaromaticum was isolated from abattoir E at all three time intervals (Fig. 5). At one week, *C. maltaromaticum* and *Bacillus* were present in larger numbers than *C. divergens, Pseudomnas* and S*taphylococcus*. At week-8, *C. maltaromaticum* dominated isolates at 70%, with smaller proportions of *Pseudomonas* and *Serratia*. High diversity was observed at week-30.

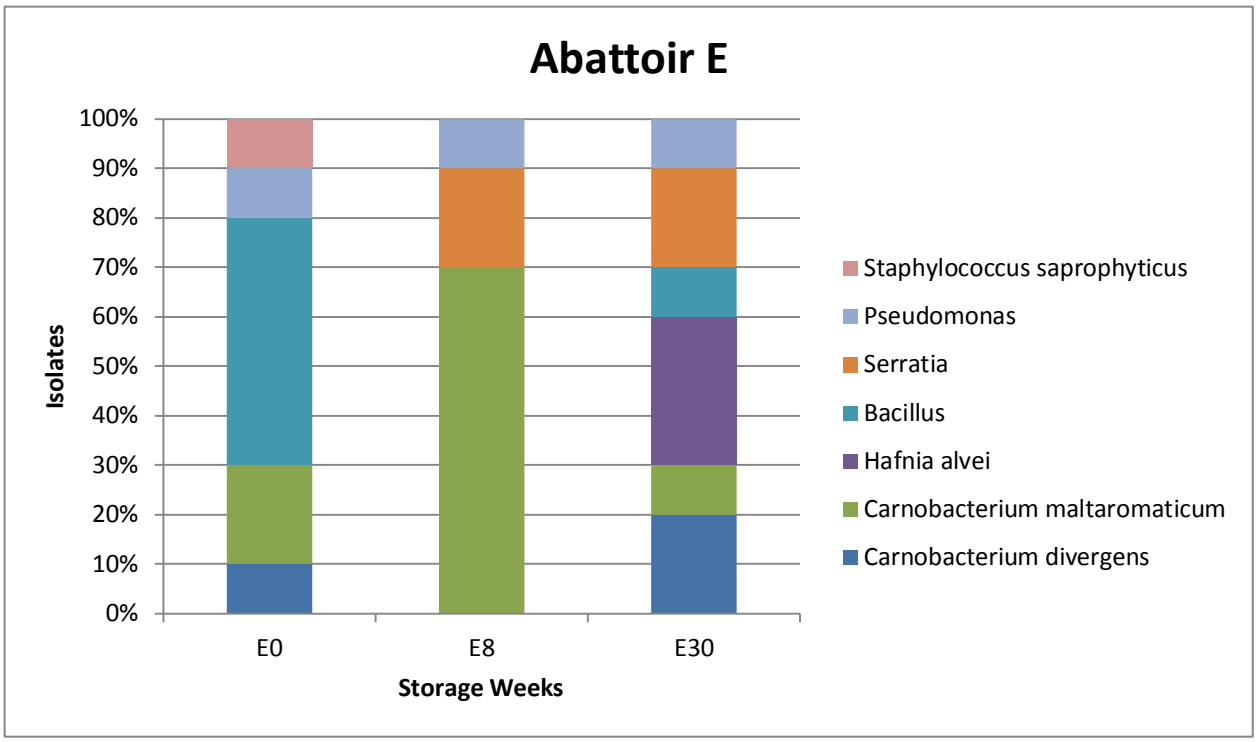

Figure 5. Abattoir E isolates at 1, 8 and 30 weeks.

Abattoir F

Abattoir F isolates were unique compared to other abattoirs. There was a very high proportion (80%) of *C. maltaromaticum* at week-1, 20% at week-8 and 40% at week-30 (Fig. 6). LAB constituted 90% of isolates at week-30.

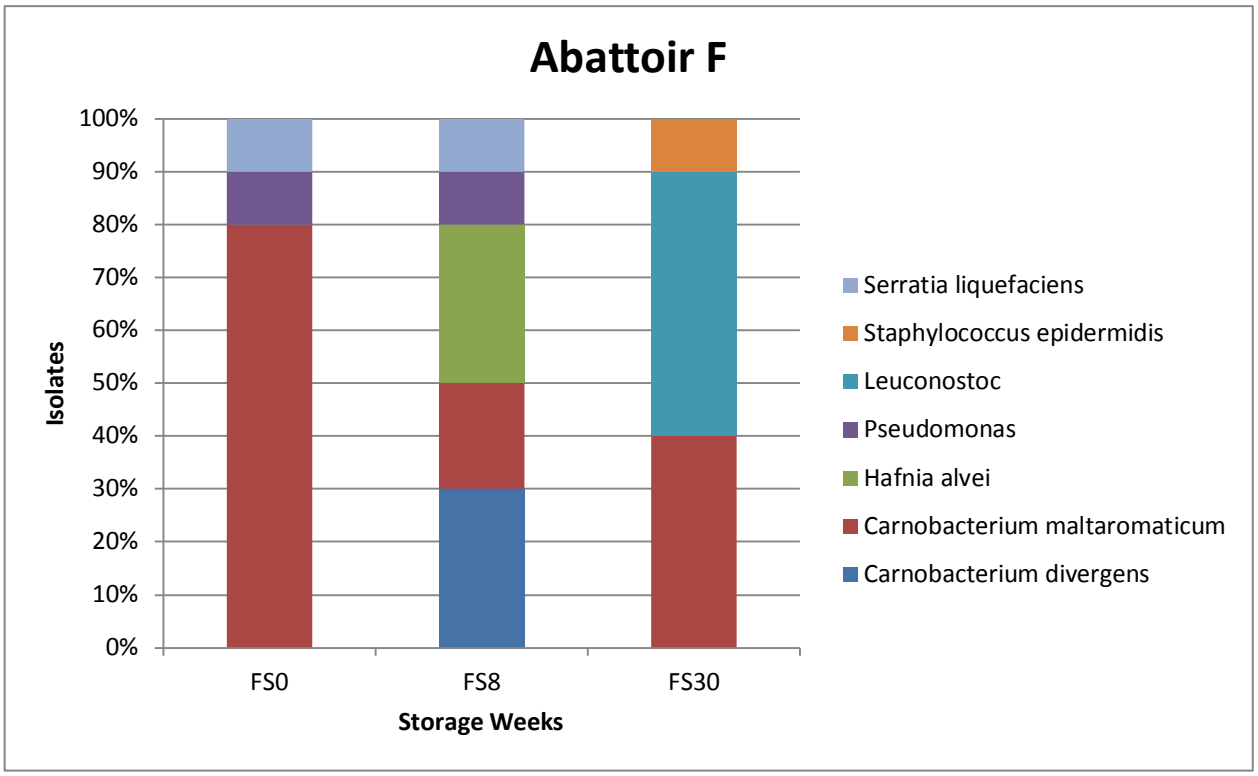

Figure 6. Abattoir F isolates at 1, 8 and 30 weeks.

Comparing isolates with the corresponding clone library (see final report A.MLA.0194), in general, the same species were observed. Most comparisons also showed similar proportions of species between the two methods. Therefore, we conclude that isolates obtained are representative of dominant culturable bacterial species in abattoir samples.

3.2 Effect of pH

Ten isolates from each abattoir, at each primal storage time interval, were incubated at 25°C for 24 h in BHI (pH 4.5, 5.0 and 5.5) and growth (>0.1 OD_{450nm}), generation time (GT) and lag time (LT) measured (Appendix 1).

A notable pattern among the isolates was that members of the Family Enterobacteriacae (e.g. *Serratia*, *Hafnia*) replicated at lower pH (4.5 and 5.0), compared to isolates in the Division Firmicutes (e.g. *Carnobacterium, Bacillus, Staphyloccus*). No associations in GT or LT were observed.

At week-1, abattoirs B, C and D had a relatively high proportion of Enterobacteriacae. Abattoir F was unique in that eight of 10 isolates were *Carnobacterium maltaromaticum*. At week-8, all isolates from abattoirs A, B and C were more sensitive to low pH (i.e. pH 4.5). Abattoir C was unique among the abattoirs as nine

out of 10 isolates were *Carnobacterium* spp., primarily *C. maltaromaticum*, and none grew at pH 4.5 or 5.0. At week-30, all abattoirs except B and E were dominated by Firmicutes, primarily lactobacilli. All isolates from abattoir D and F were Firmicutes and sensitive to pH 4.5. All abattoir D isolates were *Carnobacterium divergens*.

These results illustrate that climax microbial communities on vacuum-packaged primals differed among the six abattoirs over 30 weeks of storage. Importantly, isolates from abattoirs C, D and F were predominantly LAB, and more sensitive to lower pH. This sensitivity may be a contributing factor to the markedly lower concentration of LAB at extended storage times.

3.3 Effect of lactic acid

Lactic acid is produced within beef tissue and also by bacteria that contaminate meat surfaces. Nassos *et al*. (1983) reported that lactic acid concentrations in ground beef range from ~72-94 mM.

For the study of lactic acid, as well as for other parameters described later in this report, a subset of strains were initially tested to establish a range of parameter values for the full set of 180 beef isolates. The range chosen was one that encompassed the growth/no-growth boundary for the majority of strains, i.e. 50 to 200 mM lactic acid.

The majority of *Hafnia* and *Serratia* isolates grew in 50 and 100 mM lactic acid (Appendix 2). Some other isolates were also able to grow at 200 mM lactic acid, specifically one isolate each of *Pseudomonas* sp. (abattoir B, week-1), *Hafnia alvei* (abattoir A, week-8), *Serratia liquefaciens* (abattoir E, week-8) and two isolates of *Leuconostoc mesenteroides* (abattoir C, week-30).

The majority of lactobacilli and *Pseudomonas* isolates from week-1 and -8 did not grow in 50, 100 or 200 mM lactic acid, with the exception of abattoir F at week-8 where some isolates grew in 50 mM lactic acid. Notably, nearly all *C. maltoaromaticum* isolates did not growth in 50 mM lactic acid. This was unique for abattoir C at week-8, where 90% of isolates were *C. maltoaromaticum*. In contrast, *C. divergens* did grow in 50 mM but not higher levels of lactic acid. This latter effect was obvious at week-30 where the majority of *Carnobacterium* spp. had shifted from *C. maltoaromaticum* to *C. divergens*. Also, all isolates in abattoir D at week-30 were *C. divergens* and did not grow in 100 or 200 mM lactic acid. For abattoir F, where there were four isolates of *C. maltoaromaticum*, three unknown *Leuconostoc* spp., two *Leuconostoc carnosum*, and one *Staphylococcus epidermidis*, only one of the unknown *Leuconostoc* sp. grew in 50 mM lactic acid.

Similar to pH, no apparent trends in GT or LT were associated with abattoir and overall bacterial growth. However as for pH, the community structure of abattoir C, D and F isolates was predominantly LAB, which resulted in greater sensitivity to lactic acid.

Although one can argue that the higher concentrations of lactic acid (100-200 mM) used in these studies are at the upper end of levels expected in actual vacuum-packaged meat, we cannot excluded the possiboloty that microenvironments exist in beef tissues in which lactic acid, as well as other intrinsic factors, may be elevated and strongly influence community structure.

3.4 Effect of acetic acid

Acetic acid did not differentiate strains as observed for lactic acid and pH (Appendix 3). In general, if the strains grew, they did so at all three concentrations of acetic acid. Isolates sensitive to acetic acid were *Bacillus*, *Staphyloccus*, *Yersinia*, *Pseudomonas* and *Leuconostoc*.

3.5 Effect of glucose

The growth profiles of 10 isolates (A30f, A30g, A30h, B8b, B8c, B30b, C30b, C30e, C30f and D0a) were screened in the Bioscreen C for 24 hours at 25°C (Fig. 7). However, half of the strains initially tested (A30h, B8c, B30b, C30e and C30f) showed no growth.

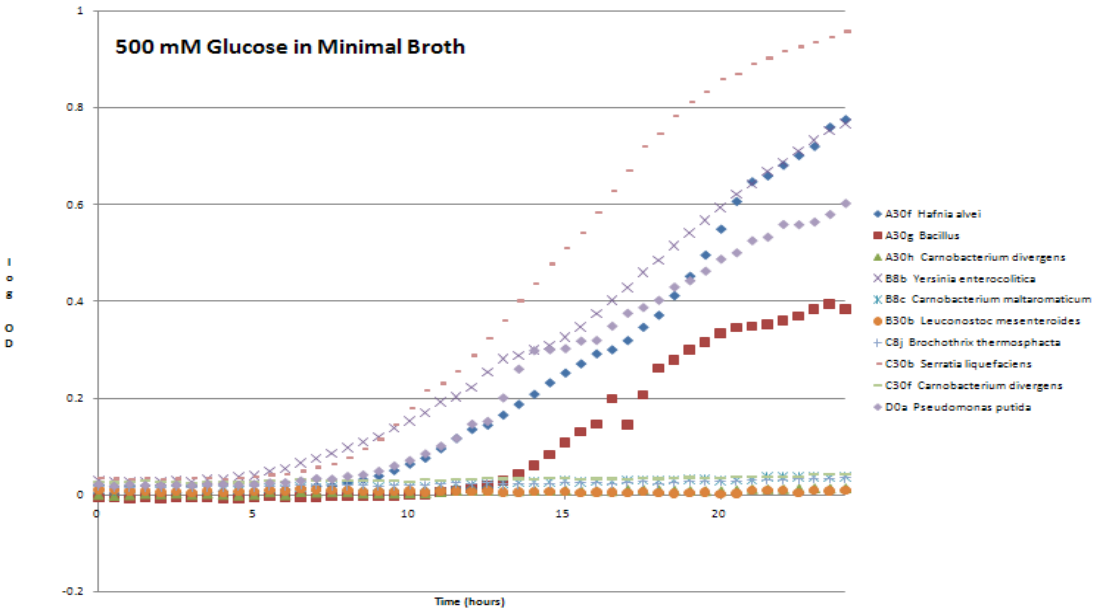

Figure 7. Growth profiles of 10 isolates in 500 mM glucose.

Further measurements indicated that the addition of a nitrogen source might be necessary for growth of all strains. Minimal broths were made with 0.25% Casamino acids (BD Difco, Australia), 0.5% Bacteriological peptone, 0.5% yeast extract (Oxoid Ltd), 0.5% tryptone (Oxoid Ltd) or 2% tryptone. Tryptone Soya broth (TSB; Oxoid Ltd) was also used. Each of these broths was used with and without the addition of filter-sterilised glucose to a final concentration of 0.9%. From this screening, 2% tryptone was used to supplement the minimal broth.

Another screening was conducted using six different glucose concentrations (0%, 0.005%, 0.01%, 0.05%, 0.1% and 1%). From this work, all 180 isolates were screened in minimal broth containing 2% tryptone and 0.005%, 0.05% or 1% glucose for 24 hours at 25°C in the Bioscreen C.

A clear species-specific pattern was observed for the effect of glucose on the bacterial isolates, however, there was no apparent association with specific abattoirs. In all cases, *Carnobacterium* spp. isolates from week-1 and -30 storage did not grow

to high densities in 0.005% glucose, as observed for *Hafnia*, *Serratia* and *Pseudomonas* spp. (Table 1).

Table 1. The percentage of isolates for individual genera that produced an OD_{540} greater than 0.9 in the minimum concentration of glucose (0.005%) in 24 hours at 25°C.

		% isolates for specific genera					
		A	**B**	**C**	**D**	**E**	**F**
1 week	Carnobacterium	0	0	0	0	0	0
	Hafnia	100			100		
	Bacillus	0				0	
	Serratia		100	100	100		100
	Leuconostoc						
	Pseudomonas		100	100	0	100	0
	Staphylococcus						
	Brochothrix	0					
30 weeks	Carnobacterium	0	0	0	0	0	0
	Hafnia	100				100	
	Bacillus	0	0			0	
	Serratia		100	100		100	
	Leuconostoc		0	0			0
	Pseudomonas					100	
	Staphylococcus						0
	Brochothrix						

In contrast, the growth of *Carnobacterium*. was markedly stimulated by increased levels of glucose, whereas *Hafnia*, *Serratia* and *Pseudomonas* were not. Table 2 shows the percentage of isolates within specific genera that displayed greater than a 0.3 OD_{540} increase after 24 hours incubation at 25°C.

Table 2. The percentage of isolates for individual genera where the difference in OD_{540} between 0.005 and 1.0% glucose was greater than 0.3 in 24 hours at 25°C.

		% isolates for specific genera					
		A	**B**	**C**	**D**	**E**	**F**
1 week	Carnobacterium	83	100	100	100	100	88
	Hafnia	0			0		
	Bacillus	0				0	
	Serratia		0	0	0		0
	Leuconostoc						
	Pseudomonas		0	0	0	0	0
	Staphylococcus						
	Brochothrix	0					
30 weeks	Carnobacterium	71	100	100	80	67	75
	Hafnia	0				0	
	Bacillus	0	0			0	
	Serratia		0	0		0	
	Leuconostoc		0	100			0
	Pseudomonas					0	
	Staphylococcus						0
	Brochothrix						

3.6 Effect of low temperature

Appendices 4, 5 and 6 show the change in OD_{540} for abattoir isolates after storage at -2.5, -1 and 1°C for five weeks. Similar profiles were observed among isolates for specific genera/species, regardless of the abattoir.

At -2.5°C, relatively high growth (~0.8-1.0 OD_{540}) was observed for *Brochothrix* and *Pseudomonas* spp. *Carnobacterium*, *Yersinia*, *Bacillus* showed intermediate growth (~0.4-0.7 OD_{540}). Low or no detectable growth (<0.3 OD_{540}) was observed for *Hafnia*, *Serratia*, *Staphylococcus* and *Leuconostoc* spp. Although exceptional, a few *Pseudomonas*, *Carnobacterium* and *Bacillus* isolates also displayed low growth.

Notably at -1°C, no or low growth was observed for *Leuconostoc*, *Bacillus* and *Staphylococcus*, and also for some *Hafnia* isolates. The vast majority of isolates grew at 1°C, with exceptions for a few *Leuconostoc* and *Bacillus* isolates.

Abattoirs displaying a higher proportion of the same genera, such as *Carnobacterium* in abattoirs C and E at week-8, showed a narrower distribution in growth profiles. This was more pronounced at 30 weeks where there is less species diversity.

3.7 Bacterial inhibition

Inhibitory interactions among isolates from each abattoir were characterised first on agar, where a target isolate was spread-plated and then the potential effector strains spotted onto the surface. After overnight incubation, zones of inhibition were recorded.

Table 3 shows the number of isolates from each abattoir that inhibited any of the 30 isolates (10 isolates per time interval) from the same abattoir, as well as inhibitions between individual *Carnobacterium* isolates.

Abattoirs C, D, E and F were notable by having greater than 30 interactions (out of potentially 830 interactions per abattoir). In addition, there were 35 and 12 *Carnobacterium-Carnobacterium* isolate interactions for abattoirs D and C, respectively, compared to 3, 7, 4 and 0 interactions for abattoirs A, B, E and F, respectively. Abattoirs C, D, E and F also had more *Serratia, Pseudomonas, Hafnia, Bacillus* and *Staphylococcus* isolates that inhibited other species.

Table 3. Inhibitory interactions among isolates from six abattoirs. In cases of 10 or more interactions among isolates, data cells are highlighted in yellow colour.

	Abattoir					
Inhibition by:	A	B	C	D	E	F
Brochothrix	9		2			
Carnobacterium	4	3	1	1	20	5
Serratia			12		13	
Pseudomonas		2	4	54	4	11
Yersinia	2	1				
Rahnella		1				
Leuconostoc		2				2
Hafnia	1			1	16	
Bacillus	2				18	
Staphylococcus						14
Carno-Carno inhibition	3	7	12	35	4	
	21	**16**	**31**	**91**	**75**	**32**

Table 4 summarises interactions among isolates from different weeks. For example, when testing the 10 isolates from abattoir A at week-1 against 10 isolates from abattoir A at weeks 1, 8 and 30, there were three interactions for week-1, one interaction for week-8 and three interactions for week-30. A similar comparison for abattoir D week-1 isolates showed 10, 31 and 40 interactions for weeks-1, 8 and 30, respectively.

Table 4. A summary of inhibitory interactions among isolates from the six abattoirs at 1, 8 and 30 weeks. Values in cells indicate the number of inhibitory interactions among 10 isolates from each week interval (column) tested against 10 isolates from each week interval (row). Greater than 10 interactions are highlighted in yellow.

		1	8	30
A	1	3	2	1
	8	1	1	3
	30	3	5	2
		7	8	6

B	1	1	0	1
	8	4	0	0
	30	6	2	2
		11	2	3

C	1	10	0	3
	8	0	1	1
	30	5	7	4
		15	8	8

D	1	10	0	0
	8	31	0	1
	30	40	0	9
		81	0	10

E	1	28	3	17
	8	2	7	0
	30	6	3	9
		36	13	26

F	1	2	0	4
	8	6	0	5
	30	3	2	10
		11	2	19

Figure 8 ranks the bacterial genera by the number of inhibitory reactions with other isolates. *Carnobacterium* and *Pseudomonas* spp. were the dominant species that inhibited isolates within or outside of the genera.

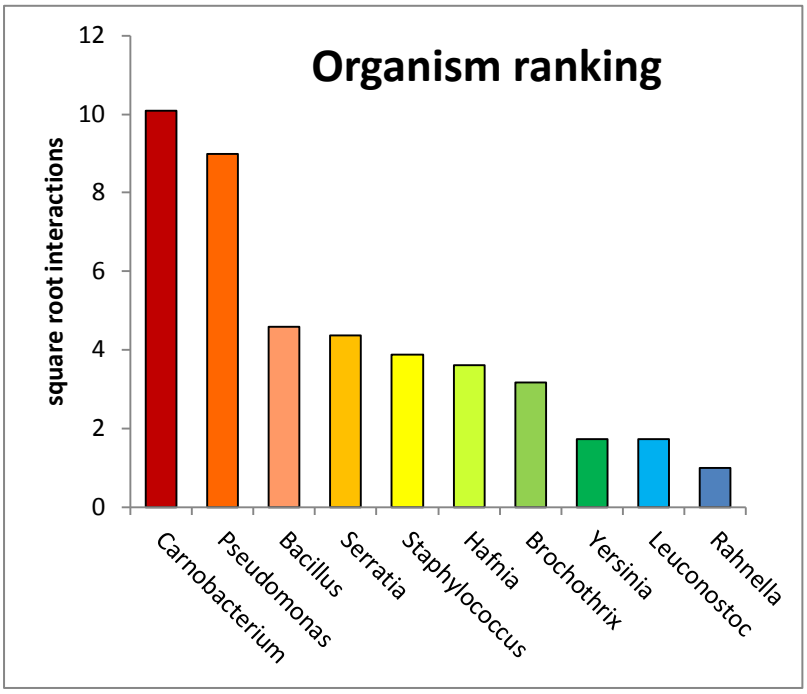

Figure 8. Ranking of bacterial genera based on number of interactions (square root) with other isolates.

There were more interactions among isolates from abattoir E than other abattoirs (Fig. 9). This was followed by abattoir D, C, F, A and B.

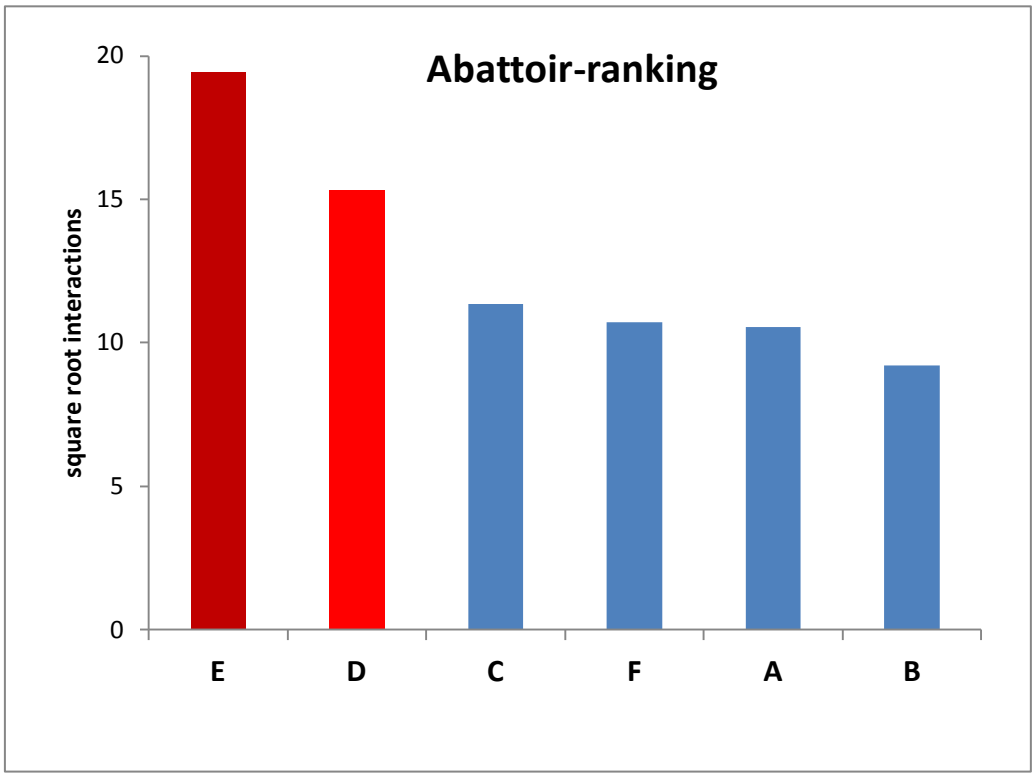

Figure 9. Ranking of abattoirs based on number of bacterial interactions (square root) among isolates.

A similar ranking of interactions among isolates at specific weeks was done, showing that the largest number of interactions occurred between isolates from weeks1-30, followed by weeks 1-1, weeks-30-30 and weeks-1-8.

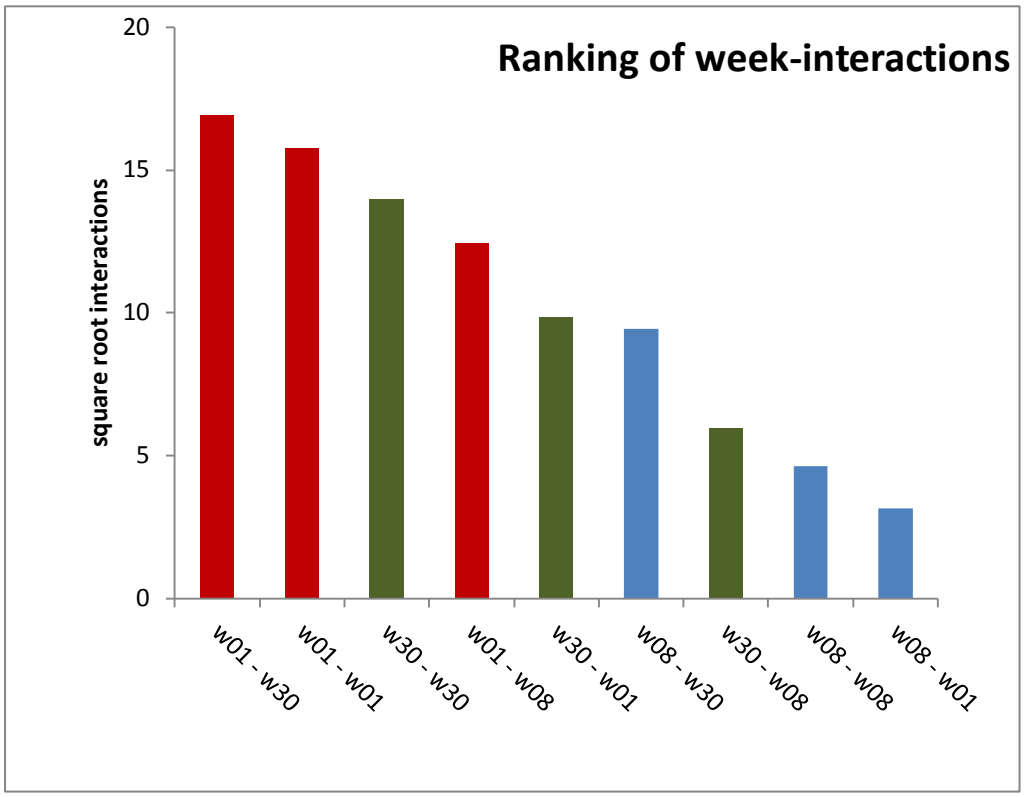

Figure 10. Ranking of week-week interactions based on number of interactions (square root) among isolates.

Next, data for isolates (only the subset showing inhibitory properties) were analysed with a network analysis tool (Cytoscape) to visual interactions among genera/species for each abattoir.

Figures 11-16 show interactions among the isolates. The diameter of the circles (node) and the lines (edges) are proportional to the number of interacting isolates. Arrows indicate the direction of the inhibition; arrows that point back to the same genera/species indicate inhibition by isolates within the same genera/species. To normalise the analysis, the same bacterial genera are shown on each plot. Nodes without associated edges are bacterial genera that were represented in that specific abattoir.

Figure 17 shows interactions for all abattoirs. As described above, this plot demonstrates the dominant inhibitory effects of *C. maltaromaticum*, *C. divergens* and *Pseudomonas* spp.

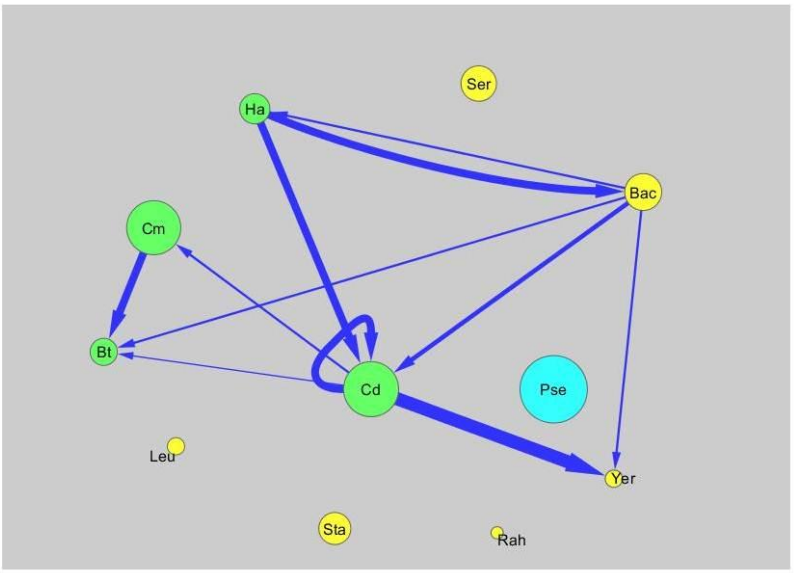

Figure 11. Interactions among bacterial isolates from abattoir A.

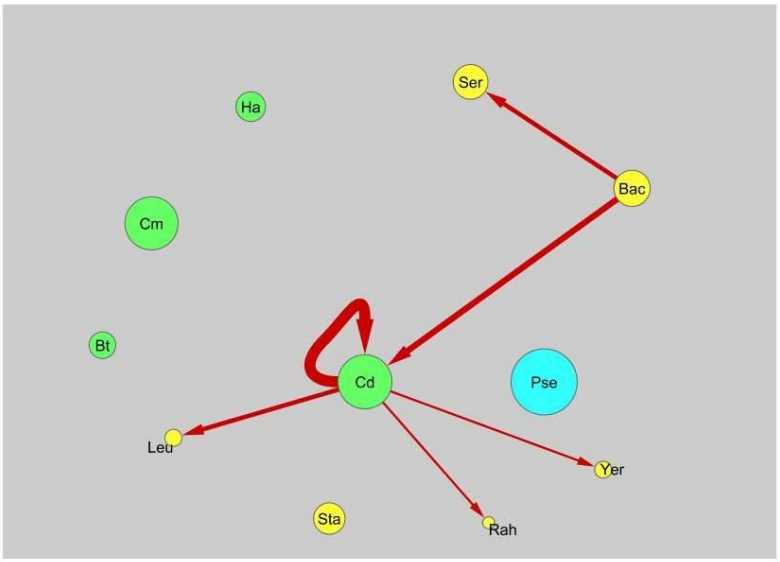

Figure 12. Interactions among bacterial isolates from abattoir B.

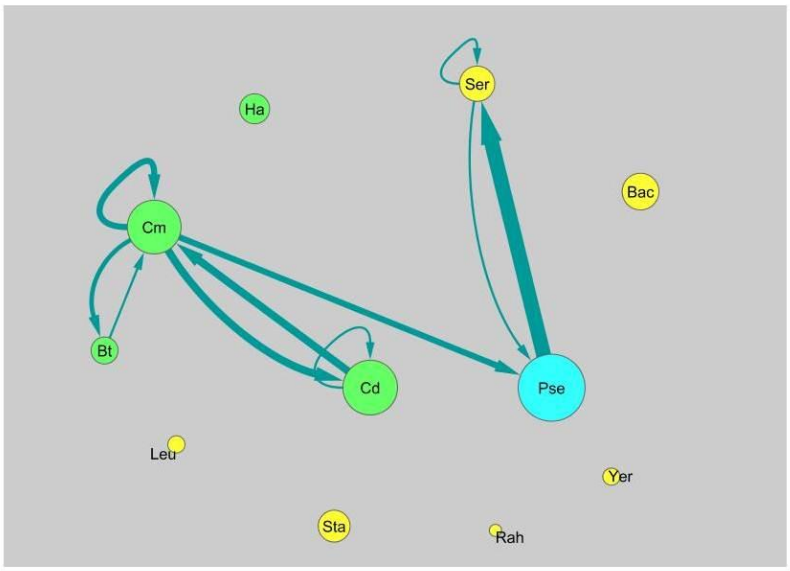

Figure 13. Interactions among bacterial isolates from abattoir C.

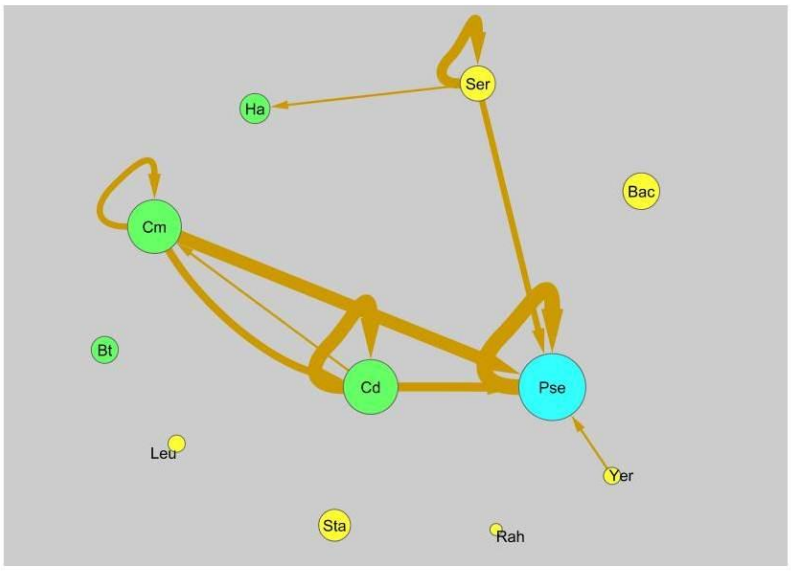

Figure 14. Interactions among bacterial isolates from abattoir D.

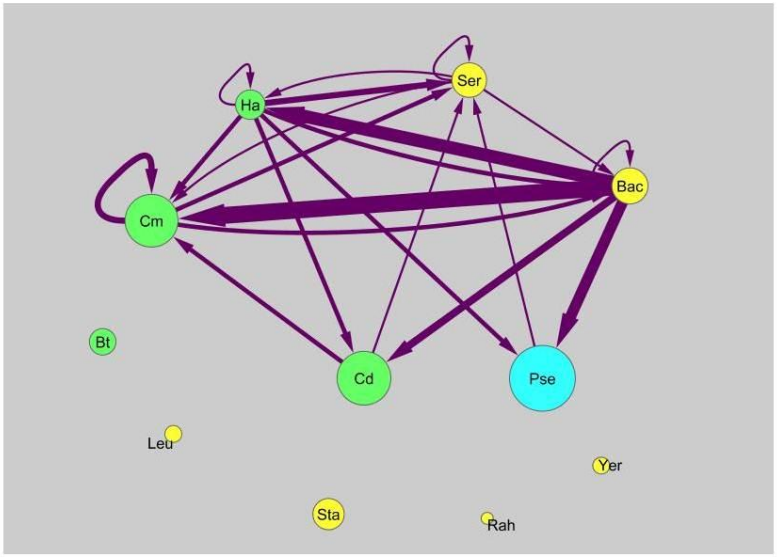

Figure 15. Interactions among bacterial isolates from abattoir E.

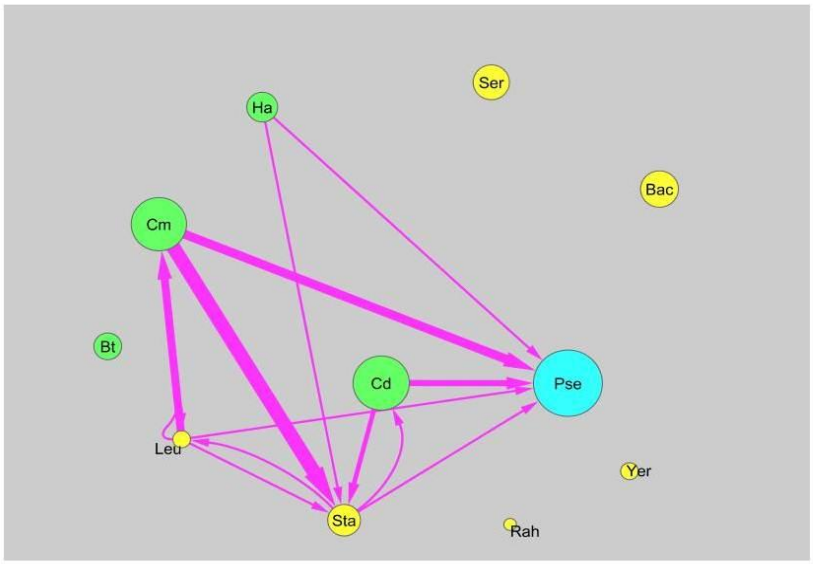

Figure 16. Interactions among bacterial isolates from abattoir F.

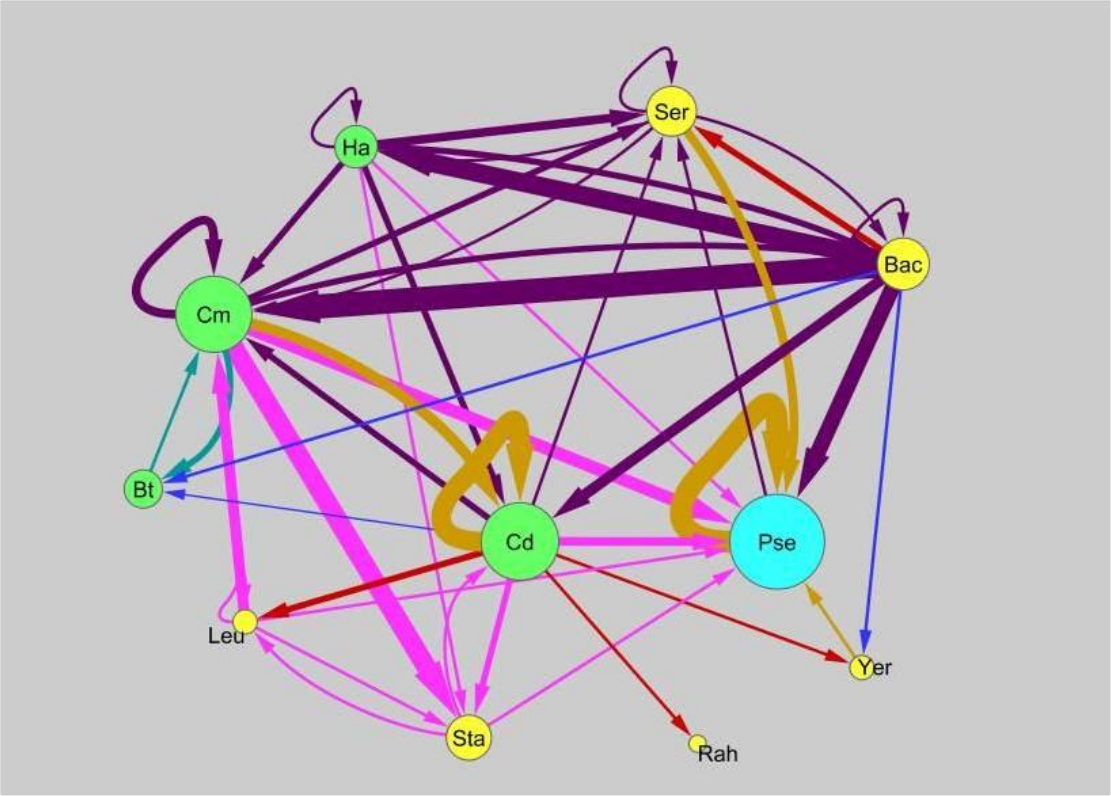

Figure 17. Interactions among all bacterial isolates from all abattoirs.

In a separate phase of the inhibition studies, the strength of inhibition between selected isolates was measured in the Bioscreen C by making cell-free supernantants of overnight cultures, placing 2-fold serial dilutions of the supernatants in the Bioscreen plate and then adding the target strain to each well. The OD of each well was measured after 24 hours at 25°C, and then growth rates determined. The last dilution that reduced the growth rate compared to the negative control was recorded.

Results showed that the greatest inhibitory effect was seen for interactions within the same species, indicating bacteriocins as likely factors. For example, the titre for the average *C. divergens-C. divergens* and *C. maltaromaticum-C. maltaromaticum* interaction was 242 and 256, respectively. The titre for the *C. maltaromaticum-C. divergens* interaction was 107. In contrast, much lower inhibitory effects were observed for intergenera interactions.

Finally, we found that inhibitory interactions among isolates varied depending on the test for format. Specifically, *Psuedomonas* inhibited more isolates in the agar format compared to the Bioscreen. This indicates that *Psuedomonas* may primarily exert an inhibitory effect via cell-contact, a property that would not be seen using cell-free supernatants in the Biosecreen.

Table 9. Average titre of inhibition produced by isolates.

		Target					
		C. divergens	C. maltaromaticum	Hafnia alvei	B. subtilis	Pseudomonas	Serratia proteamaculans
Inhibitor	C. divergens	242	2	2	2		
	C. maltaromaticum	107	256	2	8		
	Bacillus spp.			48			
	Pseudomonas spp.	3			2	7	3

4 Conclusions and recommendations

This comprehensive study shows that strains of bacteria on VP primals from abattoirs with low bacterial growth are more sensitive to pH, lactic acid and to low concentrations of glucose. Importantly, these abattoirs have a higher proportion of strains that produce inhibitory compounds against other bacteria, with the greatest effects against bacteria of the same species. These findings also indicate that early suppression of Enterobacteriacae and *Pseudomonas* may be a key step for successful dominance of 'friendly' LAB.

Additional studies are recommended to achieve full benefits to the industry. These include characterising, modelling and validating bacterial interactions under dynamic conditions that are relevant to commercial operations, and identifying the factors that mediate bacterial inhibition. With this information, it would be possible to recommend and design production controls that maintain bacterial flora that promote extended shelf-life and quality.

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6 Appendices

Appendix 1. Growth parameters for bacterial isolates tested at pH 4.5, 5.0 and 5.5, 25°C for 24 hours in TSB. A value of 1 in the "Growth" column indicates that the optical density of the TSB medium increase by more than 0.1 unit. GT and Lag indicate generation time and lag time in hours.

Abattoir	Time (week)	Genus/species	pH 4.5			pH 5.0			pH 5.5		
			Growth	GT	Lag	Growth	GT	Lag	Growth	GT	Lag
A	1	Carnobacterium divergens							1	5.0	14.6
		Brochothrix thermosphacta									
		Carnobacterium divergens							1	5.4	14.8
		Carnobacterium divergens							1	5.3	14.3
		Carnobacterium maltaromaticum							1	8.3	18.3
		Carnobacterium maltaromaticum							1	3.6	7.3
		Hafnia alvei							1	3.2	7.1
		Bacillus									
		Carnobacterium maltaromaticum							1	10.5	18.3
		Hafnia alvei				1	4.3	9.7	1	3.0	7.6

		% showing growth or average				**1**	**4.3**	**9.7**	**8**	**5.5**	**12.8**
		Serratia proteamaculans				1	5.2	11.0	1	3.6	7.3
		Serratia proteamaculans	1	8.0	15.7	1	5.5	11.3	1	3.6	7.4
		Carnobacterium divergens							1	3.6	7.3
		Serratia proteamaculans				1	5.0	11.8	1	3.6	7.4
		Carnobacterium divergens							1	3.6	7.5
B	1	Carnobacterium divergens							1	3.8	7.4
		Serratia liquefaciens	1	8.3	18.4	1	4.7	9.5	1	3.8	7.2
		Serratia				1	5.6	13.0	1	3.6	7.4
		Pseudomonas				1	4.8	19.0	1	5.9	16.0
		Pseudomonas							1	5.0	15.8
		% showing growth or average	**2**	**8.1**	**17.1**	**6**	**5.1**	**12.6**	**10**	**4.0**	**9.1**
		Carnobacterium divergens							1	5.4	17.9
		Serratia proteamaculans				1	4.8	12.7	1	3.3	5.3

		Pseudomonas fluorescens							1	3.1	14.4
		Serratia proteamaculans				1	5.4	12.6	1	3.5	5.1
		Serratia				1	5.2	12.8	1	3.5	5.0
C	1	Pseudomonas							1	3.2	14.9
		Serratia proteamaculans				1	5.1	13.1	1	3.0	5.5
		Pseudomonas							1	3.2	15.6
		Pseudomonas putida							1	2.9	16.0
		Serratia proteamaculans	1	6.7	18.5	1	5.0	12.5	1	3.0	7.3
		% showing growth or average	**1**	**6.7**	**18.5**	**5**	**5.1**	**12.7**	**10**	**3.4**	**10.7**
		Pseudomonas putida									
		Pseudomonas putida									
		Serratia proteamaculans	1	6.5	15.5	1	6.0	4.3	1	2.7	2.3
		Serratia sp.	1	6.6	15.9	1	6.1	3.7	1	2.2	3.3
		Serratia sp.	1	6.8	15.4	1	5.0	4.8	1	2.3	3.1
D	1	Hafnia alvei	1	6.3	12.1	1	3.7	5.0	1	2.6	3.1

		Pseudomonas lundensis							1	2.4	4.2
		Carnobacterium divergens							1	7.5	10.7
		Carnobacterium maltaromaticum							1	7.2	10.5
		Pseudomonas putida									
		% showing growth or average	**4**	**6.6**	**14.7**	**4**	**5.2**	**4.5**	**7**	**3.8**	**5.3**
		Carnobacterium maltaromaticum							1	5.0	10.9
		Bacillus subtilis									
		Staphylococcus saprophyticus									
		Carnobacterium maltaromaticum							1	5.0	11.3
		Bacillus subtilis									
E	1	Pseudomonas									
		Bacillus subtilis									
		Bacillus subtilis									
		Bacillus subtilis									
		Carnobacterium divergens							1	4.0	8.6

		% showing growth or average							**3**	**4.7**	**10.3**
F	1	Carnobacterium maltaromaticum							1	5.6	11.6
		Pseudomonas lundensis							1	3.4	4.7
		Carnobacterium maltaromaticum							1	5.1	12.1
		Carnobacterium maltaromaticum							1	6.1	12.3
		Carnobacterium maltaromaticum							1	6.1	12.3
		Carnobacterium maltaromaticum							1	5.9	12.6
		Carnobacterium maltaromaticum							1	5.4	11.8
		Carnobacterium maltaromaticum							1	5.7	12.7
		Carnobacterium maltaromaticum							1	4.8	11.7
		Serratia liquefaciens	1	6.0	14.8	1	4.4	7.0	1	3.1	3.5
		% showing growth or average	**1**	**6.0**	**14.8**	**1**	**4.4**	**7.0**	**10**	**5.1**	**10.5**
		Yersinia intermedia				1	8.4	14.7	1	5.9	17.6
		Carnobacterium maltaromaticum							1	6.5	18.3

		Yersina itermedia				1	8.3	19.0			
		Yersinia frederiksenii				1	9.1	10.3			
		Hafnia alvei				1	4.1	14.1	1	3.2	9.0
A	8	Brochothrix thermosphacta							1	3.4	20.2
		Carnobacterium divergens							1	7.8	19.4
		Yersinia frederiksenii				1	10.3	10.6			
		Hafnia alvei				1	4.2	11.7	1	3.1	7.8
		Carnobacterium divergens				1	7.3	17.5	1	3.2	13.4
		% showing growth or average				**7**	**7.4**	**14.0**	**7**	**4.7**	**15.1**
		Carnobacterium divergens				1	7.8	18.0	1	3.7	13.4
		Yersinia enterocolitica				1	8.7	11.1	1	6.8	11.2
		Carnobacterium maltaromaticum							1	8.4	17.6
		Carnobacterium divergens				1	8.4	17.5	1	3.9	13.5
		Hafnia alvei				1	6.6	13.6	1	5.0	9.9
B	8	Rahnella							1	3.6	9.1

		Hafnia alvei				1	7.4	13.3	1	5.4	6.8
		Carnobacterium divergens				1	4.2	13.3	1	4.0	11.8
		Carnobacterium divergens							1	4.0	11.6
		Pseudomonas sp.							1	2.1	16.2
		% showing growth or average				**6**	**7.2**	**14.5**	**10**	**4.7**	**12.1**
C	8	Carnobacterium divergens							1	5.5	15.8
		Carnobacterium maltaromaticum							1	5.8	13.3
		Carnobacterium maltaromaticum							1	6.5	15.0
		Carnobacterium maltaromaticum							1	6.2	12.5
		Carnobacterium maltaromaticum							1	4.5	12.1
		Carnobacterium maltaromaticum							1	6.1	15.3
		Carnobacterium maltaromaticum							1	5.5	12.6
		Carnobacterium maltaromaticum							1	6.2	14.3
		Carnobacterium maltaromaticum							1	5.6	15.9
		Brochothrix thermosphacta				1	9.8	19.7	1	1.9	16.6

		% showing growth or average				**1**	**9.8**	**19.7**	**10**	**5.4**	**14.3**
D	8	Serratia	1	5.9	90.0	1	3.5	7.1	1	2.2	3.4
		Carnobacterium maltaromaticum				1	10.0	8.4	1	6.0	7.5
		Carnobacterium maltaromaticum							1	6.0	7.5
		Pseudomonas							1	5.3	11.2
		Carnobacterium maltaromaticum							1	3.6	9.1
		Pseudomonas lundensis							1	5.5	11.1
		Pseudomonas lundensis							1	5.2	8.7
		Carnobacterium maltaromaticum							1	6.1	7.7
		Carnobacterium divergens				1	4.2	14.3	1	5.2	11.2
		Serratia	1	6.1	14.8	1	3.0	7.2	1	3.2	7.7
		% showing growth or average	**2**	**6.0**	**52.4**	**4**	**5.2**	**9.3**	**10**	**4.8**	**8.5**
		Carnobacterium maltaromaticum							1	6.0	10.8
		Carnobacterium maltaromaticum							1	6.1	11.0

		Serratia	1	7.9	16.0	1	6.0	8.1	1	2.5	4.3
		Carnobacterium maltaromaticum							1	5.8	11.2
		Pseudomonas							1	1.7	15.4
E	8	Carnobacterium maltaromaticum							1	6.1	12.4
		Carnobacterium maltaromaticum							1	5.6	12.5
		Carnobacterium maltaromaticum							1	5.8	12.6
		Serratia liquefaciens	1	6.3	15.8	1	4.2	6.9	1	2.5	4.1
		Carnobacterium maltaromaticum							1	5.7	12.3
		% showing growth or average	**2**	**7.1**	**15.9**	**2**	**5.1**	**7.5**	**10**	**4.8**	**10.7**
		Carnobacterium maltaromaticum							1	5.5	11.9
		Pseudomonas							1	1.9	12.6
		Hafnia alvei	1	5.5	12.7	1	3.9	6.3	1	2.9	3.9
		Carnobacterium divergens				1	7.1	14.6	1	3.4	9.6
		Serratia liquefaciens	1	6.5	14.2	1	4.5	7.7	1	2.6	4.6
F	8	Carnobacterium divergens				1	7.1	15.1	1	3.6	9.5

		Carnobacterium maltaromaticum							1	5.0	12.2
		Hafnia alvei	1	5.4	12.6	1	3.8	6.7	1	2.6	4.4
		Hafnia alvei	1	5.3	13.1	1	3.7	7.2	1	2.8	4.3
		Carnobacterium divergens				1	7.2	15.3	1	3.5	9.5
		% showing growth or average	**4**	**5.7**	**13.2**	**7**	**5.3**	**10.4**	**10**	**3.4**	**8.2**
		Carnobacterium divergens					103.8	14.3	1	5.5	15.6
		Carnobacterium divergens					32.0	17.0	1	4.8	12.4
		Carnobacterium divergens					66.9	15.5	1	5.9	14.4
		Carnobacterium divergens					64.0	15.6	1	4.8	15.4
		Carnobacterium divergens					27.9	18.7	1	6.1	15.4
A	30	Hafnia alvei	1	17.4	16.1	1	4.4	9.4	1	3.1	7.7
		Bacillus				1	4.1	17.7		13.4	13.2
		Carnobacterium divergens							1	5.2	13.5
		Carnobacterium divergens					30.1	17.1	1	4.6	13.4
		Bacillus subtilis	1	18.2	17.7	1	4.9	10.1	1	9.7	15.3

		% showing growth or average	**2**	**17.8**	**16.9**	**3**	**37.6**	**15.0**	**9**	**6.3**	**13.6**
		Bacillus subtilis									
		Leuconostoc mesenteroides					29.2	16.9	1	11.7	17.0
		Serratia proteamaculans				1	5.7	12.7	1	3.4	5.2
		Carnobacterium divergens	1	18.8	18.1	1	4.9	12.4	1	3.5	8.5
		Bacillus subtilis				1	5.1	14.5			
B	30	Serratia	1	8.6	17.2	1	4.5	10.0	1	7.0	0.1
		Serratia	1	8.1	17.5	1	5.1	8.8	1	3.3	6.6
		Serratia	1	8.3	1.7	1	5.4	8.8	1	3.5	6.4
		Serratia	1	8.3	17.5	1	4.9	9.5	1	13.3	15.6
		Serratia	1	8.0	17.4	1	5.7	12.1	1	13.0	15.2
		% showing growth or average	**6**	**10.0**	**14.9**	**8**	**7.8**	**11.8**	**8**	**7.3**	**9.3**
		Serratia				1	5.8	12.6	1	3.5	4.6
		Serratia liquefaciens				1	5.5	12.4	1	3.7	4.2

		Leuconostoc mesenteroides				1	3.5	1.2	1	9.9	12.1
		Serratia	1	7.6	18.2	1	5.6	13.6	1	3.4	7.0
		Carnobacterium divergens				1	16.9	17.3	1	4.2	12.5
C	30	Carnobacterium divergens					77.2	16.6	1	4.2	11.1
		Leuconostoc mesenteroides					43.0	14.0	1	9.7	13.4
		Carnobacterium maltaromaticum							1	5.6	13.9
		Carnobacterium divergens					32.0	14.2	1	4.1	10.9
		Carnobacterium maltaromaticum							1	7.0	12.7
		% showing growth or average	**1**	**7.6**	**18.2**	**5**	**23.7**	**12.7**	**10**	**5.5**	**10.2**
		Carnobacterium divergens				1	7.4	14.7	1	3.6	7.8
		Carnobacterium divergens				1	8.1	14.8	1	3.7	7.9
		Carnobacterium divergens				1	8.1	15.6	1	3.8	7.9
		Carnobacterium divergens				1	9.0	14.1	1	3.5	8.3
		Carnobacterium divergens				1	8.2	15.4	1	3.8	7.9
D	30	Carnobacterium divergens				1	14.7	16.5	1	4.1	9.7

		Carnobacterium divergens				1	8.5	14.2	1	3.5	8.3
		Carnobacterium divergens				1	8.3	13.6	1	3.6	8.1
		Carnobacterium divergens				1	8.9	14.0	1	3.8	8.1
		Carnobacterium divergens				1	8.8	13.6	1	3.4	8.5
		% showing growth or average				**10**	**9.0**	**14.7**	**10**	**3.7**	**8.2**
		Carnobacterium divergens				1	8.9	14.7	1	3.9	9.6
		Carnobacterium divergens				1	8.5	14.4	1	3.8	9.6
		Bacillus									
		Hafnia alvei	1	6.1	11.6	1	6.1	3.9	1	2.7	0.2
		Hafnia alvei	1	5.8	12.6	1	3.8	6.8	1	2.7	4.0
E	30	Hafnia alvei	1	5.9	12.8	1	4.0	6.2	1	2.8	3.8
		Pseudomonas				1	6.2	9.0	1	4.2	5.2
		Serratia	1	6.4	14.7	1	4.7	5.8	1	2.6	3.5
		Carnobacterium maltaromaticum				0	31.4	15.6	1	5.6	10.5
		Serratia	1	6.6	14.7	1	4.7	5.9	1	2.9	3.5

		% showing growth or average	**5**	**6.2**	**13.3**	**8**	**8.7**	**9.2**	**9**	**3.5**	**5.6**
F	30	Carnobacterium maltaromaticum				1	29.5	15.2	1	5.0	10.2
		Carnobacterium maltaromaticum				1	25.9	14.3	1	4.9	10.8
		Staphylococcus epidermidis									
		Carnobacterium maltaromaticum									
		Leuconostoc sp.									
		Leuconostoc sp.									
		Leuconostoc sp.									
		Leuconostoc carnosum									
		Carnobacterium maltaromaticum				**1**	30.7	15.5	**1**	4.7	10.7
		Leuconostoc carnosum									
		% showing growth or average				**3**	**28.7**	**15.0**	**3**	**4.9**	**10.5**

Appendix 2. Growth parameters for bacterial isolates tested in 50, 100 and 200 mM lactic acid, at 25°C for 24 hours in TSB.

Abattoir	Time (week)	Genus/species	50mM Lactic Acid			100mM Lactic Acid			200mM Lactic Acid		
			Growth	GT	Lag	Growth	GT	Lag	Growth	GT	Lag
A	1	Carnobacterium divergens	1	13.4	17.5						
		Brochothrix thermosphacta									
		Carnobacterium divergens	1	3.2	20.1						
		Carnobacterium divergens									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum	1	18.9	17.7						
		Hafnia alvei	1	2.7	9.1	1	5.1	19.3			
		Bacillus									
		Carnobacterium maltaromaticum									
		Hafnia alvei	1	2.9	9.5	1	3.8	20.9			
		% showing growth or average	**5**	**8.2**	**14.8**	**2**	**4.4**	**20.1**			

		Serratia proteamaculans	1	3.3	8.8	1	5.8	17.5			
		Serratia proteamaculans	1	2.3	9.0	1	5.1	17.4			
		Carnobacterium divergens	1	7.5	19.4						
		Serratia proteamaculans	1	2.6	8.8	1	5.9	18.1			
		Carnobacterium divergens									
B	1	Carnobacterium divergens									
		Serratia liquefaciens	1	3.5	8.6	1	5.3	16.7			
		Serratia	1	2.8	8.8	1	4.9	17.0			
		Pseudomonas									
		Pseudomonas	1	2.9	9.7	1	3.8	13.7	1	5.3	21.3
		% showing growth or average	**7**	**3.6**	**10.4**	**6**	**5.1**	**16.7**	**1**	**5.3**	**21.3**
		Carnobacterium divergens									
		Serratia proteamaculans	1	2.8	9.0	1	5.2	17.4			
		Pseudomonas fluorescens									
		Serratia proteamaculans	1	3.0	8.7	1	5.4	17.4			

		Serratia	1	2.9	9.5	1	4.8	16.7			
C	1	Pseudomonas									
		Serratia proteamaculans	1	2.7	8.8	1	5.7	18.3			
		Pseudomonas									
		Pseudomonas putida									
		Serratia proteamaculans	1	3.2	8.1	1	5.6	16.9			
		% showing growth or average	**5**	**2.9**	**8.8**	**5**	**5.3**	**17.3**			
		Pseudomonas putida	1	6.1	18.3						
		Pseudomonas putida	1	16.7	17.7						
		Serratia proteamaculans	1	3.0	8.6	1	6.5	15.2			
		Serratia sp.	1	3.7	7.8	1	5.9	12.8			
		Serratia sp.	1	2.8	8.4	1	5.4	14.5			
D	1	Hafnia alvei	1	2.9	8.5	1	4.6	21.8			
		Pseudomonas lundensis	1	8.7	13.7						
		Carnobacterium divergens									

		Carnobacterium maltaromaticum									
		Pseudomonas putida	1	19.0	17.2						
		% showing growth or average	**8**	**7.9**	**12.5**	**4**	**5.6**	**16.1**			
		Carnobacterium maltaromaticum									
		Bacillus subtilis									
		Staphylococcus saprophyticus									
		Carnobacterium maltaromaticum									
		Bacillus subtilis									
E	1	Pseudomonas									
		Bacillus subtilis									
		Bacillus subtilis									
		Bacillus subtilis									
		Carnobacterium divergens	1	12.8	17.4						
		% showing growth or average	**1**	**12.8**	**17.4**						

		Carnobacterium maltaromaticum									
		Pseudomonas lundensis	1	7.1	12.5						
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
F	1	Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Serratia liquefaciens	1	2.8	8.1	1	5.5	15.7			
		% showing growth or average	**2**	**4.9**	**10.3**	**1**	**5.5**	**15.7**			
		Yersinia intermedia	1	10.9	10.8						
		Carnobacterium maltaromaticum									
		Yersina itermedia	1	11.6	14.4						
		Yersinia frederiksenii	1	13.4	10.2						

		Hafnia alvei				1	3.7	18.2	1	5.3	21.3
A	8	Brochothrix thermosphacta									
		Carnobacterium divergens									
		Yersinia frederiksenii	1	4.4	8.2						
		Hafnia alvei	1	3.1	8.1	1	4.1	19.5			
		Carnobacterium divergens	1	5.8	18.1						
		% showing growth or average	**6**	**8.2**	**11.6**	**2**	**3.9**	**18.9**	**1**	**5.3**	**21.3**
		Carnobacterium divergens	1	12.4	17.4						
		Yersinia enterocolitica	1	11.5	8.3						
		Carnobacterium maltaromaticum									
		Carnobacterium divergens	1	6.8	16.9						
		Hafnia alvei	1	5.6	10.4						
B	8	Rahnella	1	7.0	9.2	1	12.1	13.2			
		Hafnia alvei	1	7.5	9.6						
		Carnobacterium divergens	1	6.4	17.1						

		Carnobacterium divergens	1	6.7	16.3						
		Pseudomonas sp.	1	3.9	21.5						
		% showing growth or average	**9**	**7.5**	**14.1**	**1**	**12.1**	**13.2**			
C	8	Carnobacterium divergens									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Brochothrix thermosphacta	1	7.2	15.9						
		% showing growth or average	**1**	**7.2**	**15.9**						

		Serratia	1	3.1	8.8	1	6.0	18.1			
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Pseudomonas									
		Carnobacterium maltaromaticum									
D	8	Pseudomonas lundensis	1	25.1	15.4						
		Pseudomonas lundensis	1	21.9	15.4						
		Carnobacterium maltaromaticum									
		Carnobacterium divergens	1	4.1	13.9						
		Serratia	1	3.1	8.1	1	5.3	18.9			
		% showing growth or average	**5**	**11.5**	**12.3**	**2**	**5.6**	**18.5**			
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Serratia	1	3.5	9.2	1	7.6	15.9			
		Carnobacterium maltaromaticum									

		Pseudomonas									
E	8	Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Serratia liquefaciens	1	3.3	8.1	1	4.0	15.0	1	10.8	21.3
		Carnobacterium maltaromaticum									
		% showing growth or average	**2**	**3.4**	**8.6**	**2**	**5.8**	**15.5**	**1**	**10.8**	**21.3**
		Carnobacterium maltaromaticum									
		Pseudomonas	1	5.8	21.4						
		Hafnia alvei	1	3.2	7.6	1	3.1	20.6			
		Carnobacterium divergens	1	6.2	12.6						
		Serratia liquefaciens	1	3.3	8.0	1	6.0	16.0			
F	8	Carnobacterium divergens	1	6.1	12.6						
		Carnobacterium maltaromaticum									
		Hafnia alvei	1	3.1	8.0	1	6.3	20.7			

		Hafnia alvei	1	3.2	8.0						
		Carnobacterium divergens	1	6.1	12.7						
		% showing growth or average	**8**	**4.6**	**11.4**	**3**	**5.1**	**19.1**			
		Carnobacterium divergens	1	8.4	12.8						
		Carnobacterium divergens	1	12.2	11.2						
		Carnobacterium divergens	1	8.1	12.6						
		Carnobacterium divergens	1	8.8	11.8						
		Carnobacterium divergens	1	8.0	12.8						
A	30	Hafnia alvei	1	3.0	7.8	1	4.1	18.6			
		Bacillus	1	5.2	17.1						
		Carnobacterium divergens	1	8.2	12.7						
		Carnobacterium divergens	1	12.0	11.5						
		Bacillus subtilis									
		% showing growth or average	**9**	**8.2**	**12.2**	**1**	**4.1**	**18.6**			

		Bacillus subtilis									
		Leuconostoc mesenteroides	1	8.9	14.9	1	18.0	14.0			
		Serratia proteamaculans	1	10.8	5.8	1	5.0	15.8			
		Carnobacterium divergens	1	5.9	12.8						
		Bacillus subtilis									
B	30	Serratia	1	3.4	6.9	1	6.3	17.2			
		Serratia	1	3.2	6.5	1	5.3	19.8			
		Serratia	1	3.0	6.8	1	5.3	18.4			
		Serratia	1	3.7	6.5						
		Serratia	1	3.2	6.6	1	5.8	19.5			
		% showing growth or average	**8**	**5.3**	**8.3**	**6**	**7.6**	**17.5**			
		Serratia	1	3.2	6.6	1	5.2	17.2			
		Serratia liquefaciens	1	3.3	6.6	1	5.7	17.5			
		Leuconostoc mesenteroides	1	16.8	11.9	1	21.2	10.6	1	32.7	10.8
		Serratia	1	4.7	6.2	1	7.5	17.3			

		Carnobacterium divergens	1	7.9	15.2						
C	30	Carnobacterium divergens	1	6.1	15.4						
		Leuconostoc mesenteroides	1	16.6	12.2	1	21.4	11.6	1	32.5	11.7
		Carnobacterium maltaromaticum									
		Carnobacterium divergens	1	6.0	15.5						
		Carnobacterium maltaromaticum									
		% showing growth or average	**8**	**8.1**	**11.2**	**5**	**12.2**	**14.8**	**2**	**32.6**	**11.2**
		Carnobacterium divergens	1	5.6	15.8						
		Carnobacterium divergens	1	6.4	15.1						
		Carnobacterium divergens	1	5.7	15.7						
		Carnobacterium divergens	1	8.0	18.4						
		Carnobacterium divergens	1	5.8	15.8						
D	30	Carnobacterium divergens	1	11.1	18.5						
		Carnobacterium divergens	1	5.7	15.2						
		Carnobacterium divergens	1	5.7	15.7						

		Carnobacterium divergens	1	6.4	14.8						
		Carnobacterium divergens	1	5.6	16.0						
		% showing growth or average	**10**	**6.6**	**16.1**						
E	30	Carnobacterium divergens	1	11.6	14.6						
		Carnobacterium divergens	1	9.2	15.0						
		Bacillus									
		Hafnia alvei	1	3.2	6.7						
		Hafnia alvei	1	3.0	7.1	1	13.9	20.1			
		Hafnia alvei	1	3.5	6.3						
		Pseudomonas	1	5.7	8.6						
		Serratia	1	2.9	6.9	1	5.6	17.1			
		Carnobacterium maltaromaticum									
		Serratia	1	3.2	6.6	1	5.1	17.7			
		% showing growth or average	**8**	**5.3**	**9.0**	**3**	**8.2**	**18.3**			

		Carnobacterium maltaromaticum									
		Carnobacterium maltaromaticum									
		Staphylococcus epidermidis									
		Carnobacterium maltaromaticum									
		Leuconostoc sp.									
F	30	Leuconostoc sp.									
		Leuconostoc sp.	1	3.0	6.7						
		Leuconostoc carnosum									
		Carnobacterium maltaromaticum									
		Leuconostoc carnosum									
		% showing growth or average	**1**	**3.0**	**6.7**						

Appendix 3. Growth parameters for bacterial isolates tested in 0.025, 0.05 and 0.1% acetic acid, at 25°C for 24 hours in TSB.

Abattoir	Time (week)	Genus/species	0.025% Acetic Acid			0.05% Acetic Acid			0.1% Acetic Acid		
			Growth	GT	Lag	Growth	GT	Lag	Growth	GT	Lag
A	1	Carnobacterium divergens	1	4.2	9.8	1	4.3	10.6	1	4.9	13.1
		Brochothrix thermosphacta	1	25.7	4.7						
		Carnobacterium divergens	1	3.9	10.5	1	4.4	11.0	1	5.4	13.1
		Carnobacterium divergens	1	4.1	10.3	1	4.2	10.6	1	5.3	12.5
		Carnobacterium maltaromaticum	1	5.8	11.9	1	5.9	13.5	1	7.9	16.8
		Carnobacterium maltaromaticum	1	4.1	10.5	1	4.0	11.3	1	5.2	13.1
		Hafnia alvei	1	2.8	4.2	1	3.0	6.1	1	4.3	9.1
		Bacillus									
		Carnobacterium maltaromaticum	1	8.4	10.9	1	8.3	15.3	1	13.6	15.9
		Hafnia alvei	1	2.6	4.8	1	3.2	6.6	1	4.4	9.3
		% showing growth or average	**9**	**6.8**	**8.6**	**8**	**4.7**	**10.6**	**8**	**6.4**	**12.8**

		Serratia proteamaculans	1	2.6	5.2	1	4.3	4.3	1	5.0	12.1
		Serratia proteamaculans	1	2.5	5.0	1	4.4	4.4	1	5.2	13.8
		Carnobacterium divergens	1	4.2	10.2	1	4.2	4.2	1	5.0	13.4
		Serratia proteamaculans	1	2.2	5.6	1	3.2	3.2	1	4.7	13.5
		Carnobacterium divergens	1	6.8	12.9	1	7.3	7.3	1	12.3	17.1
B	1	Carnobacterium divergens	1	4.1	11.5	1	6.1	6.1	1	9.4	15.3
		Serratia liquefaciens	1	3.3	4.5	1	4.5	4.5	1	5.7	11.8
		Serratia	1	2.5	5.3	1	4.6	4.6	1	5.2	12.7
		Pseudomonas									
		Pseudomonas									
		% showing growth or average	**8**	**3.5**	**7.5**	**8**	**4.8**	**4.8**	**8**	**6.6**	**13.7**
		Carnobacterium divergens	1	5.3	10.7	1	5.7	12.3	1	7.5	15.4
		Serratia proteamaculans	1	2.3	5.5	1	4.1	7.9	1	5.1	15.5
		Pseudomonas fluorescens									
		Serratia proteamaculans	1	2.9	4.9	1	4.2	7.2	1	4.8	15.0

		Serratia	1	2.4	5.4	1	4.1	7.0	1	5.2	13.4
C	1	Pseudomonas	1	3.8	20.6						
		Serratia proteamaculans	1	2.5	5.5	1	4.2	7.0	1	4.8	13.7
		Pseudomonas	1	2.4	4.3	1	3.1	6.3	1	3.9	9.5
		Pseudomonas putida	1	2.7	4.0	1	3.2	6.3	1	4.1	9.6
		Serratia proteamaculans	1	2.5	5.2	1	4.3	7.3	1	4.6	15.0
		% showing growth or average	**9**	**3.0**	**7.3**	**8**	**4.1**	**7.7**	**8**	**5.0**	**13.4**
		Pseudomonas putida	1	12.9	18.4						
		Pseudomonas putida	1	6.9	19.1						
		Serratia proteamaculans	1	3.2	3.7	1	4.4	8.0	1	4.8	15.0
		Serratia sp.	1	2.9	4.6	1	4.6	8.3	1	7.8	13.4
		Serratia sp.	1	2.8	4.7	1	4.9	7.3	1	6.9	14.1
D	1	Hafnia alvei	1	2.7	5.8	1	2.5	7.3	1	3.9	11.5
		Pseudomonas lundensis	1	2.5	4.9	1	2.4	7.4	1	3.9	11.0
		Carnobacterium divergens	1	2.9	5.2	1	3.2	6.8	1	4.0	11.5

		Carnobacterium maltaromaticum	1	6.9	12.3	1	7.7	15.2	1	14.8	16.3
		Pseudomonas putida									
		% showing growth or average	**9**	**4.9**	**8.7**	**7**	**4.2**	**8.6**	**7**	**6.6**	**13.3**
E	1	Carnobacterium maltaromaticum	1	3.6	9.9	1	5.8	12.8	1	9.6	15.3
		Bacillus subtilis									
		Staphylococcus saprophyticus									
		Carnobacterium maltaromaticum	1	5.4	11.5	1	6.4	12.9	1	8.4	16.4
		Bacillus subtilis									
		Pseudomonas									
		Bacillus subtilis									
		Bacillus subtilis									
		Bacillus subtilis									
		Carnobacterium divergens	1	3.9	14.0	1	4.2	11.7	1	4.9	14.4
		% showing growth or average	**3**	**4.3**	**11.8**	**3**	**5.5**	**12.5**	**3**	**7.6**	**15.3**

		Carnobacterium maltaromaticum	1	6.8	15.9	1	6.9	14.1	1	7.4	14.8
		Pseudomonas lundensis	1	10.0	15.5						
		Carnobacterium maltaromaticum	1	6.6	16.9	1	6.1	14.7	1	9.7	16.2
		Carnobacterium maltaromaticum	1	6.9	15.9	1	7.0	14.3	1	11.4	16.8
		Carnobacterium maltaromaticum	1	6.1	16.4	1	6.7	13.8	1	9.0	15.2
F	1	Carnobacterium maltaromaticum	1	6.6	16.2	1	7.0	14.6	1	11.0	16.9
		Carnobacterium maltaromaticum	1	6.4	16.9	1	6.2	14.5	1	10.0	16.1
		Carnobacterium maltaromaticum	1	7.2	16.3	1	7.4	14.7	1	11.9	17.0
		Carnobacterium maltaromaticum	1	6.9	17.2	1	6.7	13.6	1	8.6	14.8
		Serratia liquefaciens	1	3.6	11.2	1	4.2	11.2	1	7.4	14.6
		% showing growth or average	**10**	**6.7**	**15.8**	**9**	**6.5**	**14.0**	**9**	**9.6**	**15.8**
		Yersinia intermedia				1	12.5	17.9			
		Carnobacterium maltaromaticum	1	6.4	14.0	1	6.1	12.6	1	7.0	14.0
		Yersina itermedia									
		Yersinia frederiksenii				1	11.9	17.2			

		Hafnia alvei	1	3.2	10.1	1	3.3	12.3	1	6.7	19.5
A	8	Brochothrix thermosphacta									
		Carnobacterium divergens	1	7.6	17.5	1	8.6	13.6	1	11.1	14.9
		Yersinia frederiksenii				1	11.5	16.7			
		Hafnia alvei	1	3.0	8.9	1	3.2	9.5	1	3.7	13.9
		Carnobacterium divergens	1	3.3	12.5	1	3.4	11.0	1	3.9	12.2
		% showing growth or average	**5**	**4.7**	**12.6**	**8**	**7.6**	**13.9**	**5**	**6.5**	**14.9**
		Carnobacterium divergens	1	3.6	12.7	1	3.7	11.1	1	3.9	13.1
		Yersinia enterocolitica	1	6.6	13.8	1	7.5	14.2			
		Carnobacterium maltaromaticum	1	7.3	15.1	1	6.9	12.7	1	8.8	14.7
		Carnobacterium divergens	1	3.6	12.2	1	3.8	10.7	1	4.1	12.3
		Hafnia alvei	1	3.8	11.6	1	4.2	13.0	1	8.0	19.1
B	8	Rahnella	1	5.1	13.1	1	6.3	15.2			
		Hafnia alvei	1	4.2	11.3	1	4.3	13.1	1	7.0	18.5
		Carnobacterium divergens	1	3.5	12.3	1	3.6	10.8	1	4.0	12.3

		Carnobacterium divergens	1	3.4	12.3	1	3.6	10.9	1	4.2	11.9
		Pseudomonas sp.									
		% showing growth or average	**9**	**4.6**	**12.7**	**9**	**4.9**	**12.4**	**7**	**5.7**	**14.6**
C	8	Carnobacterium divergens	1	6.5	14.7	1	6.7	12.4	1	10.2	12.8
		Carnobacterium maltaromaticum	1	6.4	14.4	1	6.4	12.4	1	9.4	13.3
		Carnobacterium maltaromaticum	1	6.7	16.6	1	6.5	13.9	1	8.9	15.9
		Carnobacterium maltaromaticum	1	6.6	14.6	1	5.9	12.6	1	10.8	12.4
		Carnobacterium maltaromaticum	1	6.4	15.0	1	6.6	12.4	1	9.1	13.2
		Carnobacterium maltaromaticum	1	6.4	14.4	1	6.4	12.5	1	10.1	13.1
		Carnobacterium maltaromaticum	1	5.3	15.2	1	6.4	12.5	1	9.0	13.4
		Carnobacterium maltaromaticum	1	5.8	17.3	1	7.1	13.9	1	10.4	15.1
		Carnobacterium maltaromaticum	1	5.0	15.2	1	6.2	12.3	1	9.7	12.9
		Brochothrix thermosphacta	1	3.9	11.7	1	4.4	10.4	1	4.9	12.3
		% showing growth or average	**10**	**5.9**	**14.9**	**10**	**6.3**	**12.5**	**10**	**9.3**	**13.4**

		Serratia	1	3.9	9.3	1	4.3	8.0	1	5.6	15.0
		Carnobacterium maltaromaticum				1	11.4	16.2			
		Carnobacterium maltaromaticum	1	6.6	15.4	1	6.7	12.0	1	9.8	15.2
		Pseudomonas									
		Carnobacterium maltaromaticum	1	6.0	15.7	1	6.5	12.3	1	9.3	16.0
D	8	Pseudomonas lundensis									
		Pseudomonas lundensis									
		Carnobacterium maltaromaticum	1	6.0	15.7	1	6.3	12.6	1	8.9	15.8
		Carnobacterium divergens	1	3.2	11.4	1	3.2	9.6	1	3.7	12.6
		Serratia	1	3.9	10.4	1	4.3	8.1	1	5.2	14.6
		% showing growth or average	**6**	**4.9**	**13.0**	**7**	**6.1**	**11.3**	**6**	**7.1**	**14.9**
		Carnobacterium maltaromaticum	1	6.1	15.4	1	6.7	13.3	1	10.6	16.6
		Carnobacterium maltaromaticum	1	7.0	15.8	1	7.3	13.7	1	12.0	15.8
		Serratia	1	4.4	10.9	1	4.9	10.6	1	30.7	8.6
		Carnobacterium maltaromaticum	1	6.9	15.8	1	7.3	13.4	1	11.3	15.6

		Pseudomonas									
E	8	Carnobacterium maltaromaticum	1	7.0	15.4	1	7.3	13.3	1	11.5	15.4
		Carnobacterium maltaromaticum	1	6.7	15.9	1	7.0	14.0	1	12.4	17.0
		Carnobacterium maltaromaticum	1	6.8	15.9	1	7.0	13.6	1	11.1	15.8
		Serratia liquefaciens	1	4.3	11.9	1	4.6	8.8	1	4.9	13.6
		Carnobacterium maltaromaticum	1	7.1	15.6	1	7.3	13.3	1	12.2	16.0
		% showing growth or average	**9**	**6.3**	**14.7**	**9**	**6.6**	**12.7**	**9**	**13.0**	**14.9**
F	8	Carnobacterium maltaromaticum	1	5.7	15.9	1	7.2	12.0	1	10.0	15.2
		Pseudomonas									
		Hafnia alvei	1	3.0	7.3	1	3.1	6.4	1	4.1	9.5
		Carnobacterium divergens	1	3.4	11.8	1	3.7	9.9	1	4.3	12.3
		Serratia liquefaciens	1	4.3	11.6	1	4.4	8.7	1	5.0	12.3
		Carnobacterium divergens	1	3.4	12.2	1	3.6	9.9	1	4.2	12.4
		Carnobacterium maltaromaticum	1	6.6	15.5	1	6.7	12.3	1	10.3	15.1
		Hafnia alvei	1	3.1	7.6	1	3.4	6.2	1	4.3	9.1

		Hafnia alvei	1	3.1	7.7	1	3.3	6.5	1	4.2	9.6
		Carnobacterium divergens	1	3.7	12.1	1	3.6	9.8	1	4.4	11.9
		% showing growth or average	**9**	**4.0**	**11.3**	**9**	**4.3**	**9.1**	**9**	**5.7**	**11.9**
		Carnobacterium divergens	1	4.3	12.5	1	4.5	9.9	1	5.5	11.3
		Carnobacterium divergens	1	4.1	11.5	1	4.4	10.6	1	5.7	12.4
		Carnobacterium divergens	1	4.5	12.3	1	4.7	10.0	1	5.7	11.4
		Carnobacterium divergens	1	4.7	12.5	1	4.8	9.8	1	5.7	11.3
		Carnobacterium divergens	1	4.6	12.7	1	4.6	10.2	1	5.4	11.7
A	30	Hafnia alvei	0	3.1	7.5	1	3.4	7.3	1	4.5	9.0
		Bacillus	1	8.7	18.1	1	8.7	17.1	0	250.8	11.9
		Carnobacterium divergens	1	4.2	12.7	1	4.5	10.2	1	5.7	11.5
		Carnobacterium divergens	1	4.0	11.6	1	4.5	10.2	1	5.6	12.4
		Bacillus subtilis	0	3010.0	10.6	0	1505.0	18.7	0	752.5	14.6
		% showing growth or average	**8**	**305.2**	**12.2**	**9**	**154.9**	**11.4**	**8**	**104.7**	**11.8**

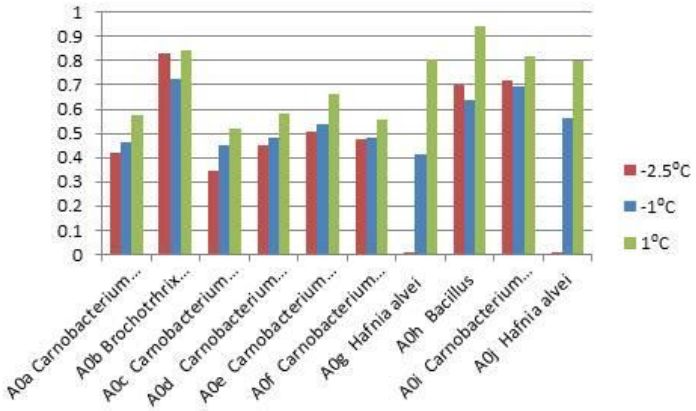
		Bacillus subtilis					3762.5	24.1			
		Leuconostoc mesenteroides	1	10.8	14.1	1	13.4	12.5	1	11.2	13.2
		Serratia proteamaculans	1	4.1	10.1	1	4.8	7.5	1	7.7	11.9
		Carnobacterium divergens	1	3.7	11.3	1	3.7	9.8	1	4.3	11.8
		Bacillus subtilis					5016.7	23.8			
B	30	Serratia	1	4.1	11.6	1	4.6	12.5	1	24.1	16.6
		Serratia	1	4.2	11.7	1	4.8	10.2	1	21.0	13.9
		Serratia	1	4.3	11.3	1	4.8	8.2	1	15.5	11.5
		Serratia	1	4.1	11.6	1	4.4	8.4	1	16.1	10.6
		Serratia	1	4.1	11.3	1	4.4	7.4	1	11.5	10.9
		% showing growth or average	**8**	**4.9**	**11.6**	**8**	**882.4**	**12.4**	**8**	**13.9**	**12.5**
		Serratia	1	4.7	11.5	1	5.1	6.6	1	15.4	11.4
		Serratia liquefaciens	1	4.2	11.2	1	4.9	6.3	1	11.3	12.3
		Leuconostoc mesenteroides	1	10.8	9.8	1	10.9	7.4	1	11.5	8.6
		Serratia	1	5.2	11.3	1	5.4	6.9	1	10.3	15.7

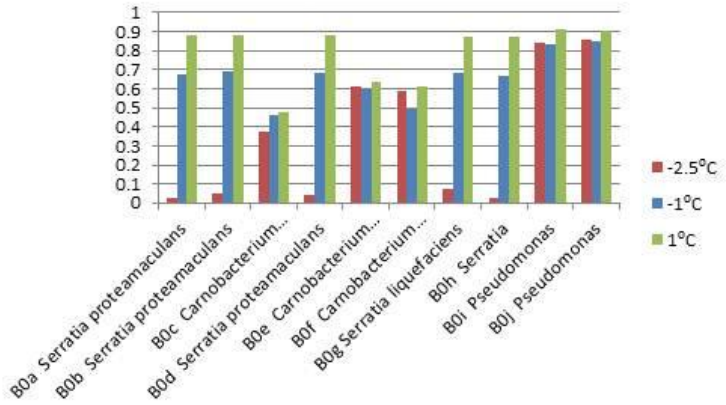
		Carnobacterium divergens	1	3.9	11.3	1	4.5	9.3	1	7.1	13.5
C	30	Carnobacterium divergens	1	3.8	12.1	1	3.8	10.2	1	4.4	13.3
		Leuconostoc mesenteroides	1	10.1	11.8	1	10.6	10.2	1	11.4	9.9
		Carnobacterium maltaromaticum	1	6.6	16.1	1	6.5	12.2	1	14.4	14.2
		Carnobacterium divergens	1	3.4	12.2	1	3.7	9.9	1	4.1	13.7
		Carnobacterium maltaromaticum	1	6.9	15.9	1	6.4	11.7	1	13.9	14.9
		% showing growth or average	**10**	**5.9**	**12.3**	**10**	**6.2**	**9.1**	**10**	**10.4**	**12.8**
		Carnobacterium divergens	1	3.8	11.6	1	3.7	9.8	1	4.8	13.7
		Carnobacterium divergens	1	3.7	11.3	1	3.7	9.7	1	4.8	12.5
		Carnobacterium divergens	1	3.8	11.6	1	3.7	9.1	1	4.3	12.2
		Carnobacterium divergens	1	5.0	14.6	1	4.5	12.1	1	15.1	10.8
		Carnobacterium divergens	1	3.7	11.8	1	3.7	9.9	1	4.5	13.6
D	30	Carnobacterium divergens	1	3.6	11.7	1	3.4	13.0	1	4.5	14.2
		Carnobacterium divergens	1	3.8	11.8	1	3.9	10.0	1	4.5	13.4
		Carnobacterium divergens	1	3.8	11.8	1	3.7	10.1	1	4.5	13.4

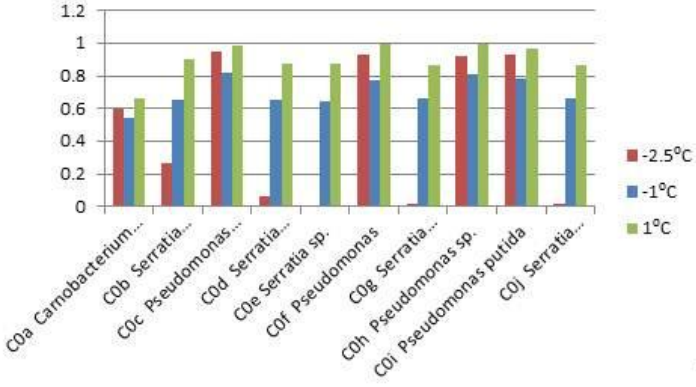
		Carnobacterium divergens	1	4.1	11.5	1	3.9	9.9	1	4.6	12.9
		Carnobacterium divergens	1	3.7	11.9	1	3.8	9.9	1	5.0	12.8
		% showing growth or average	**10**	**3.9**	**12.0**	**10**	**3.8**	**10.3**	**10**	**5.6**	**12.9**
E	30	Carnobacterium divergens	1	4.8	10.9	1	4.8	10.1	1	6.5	13.3
		Carnobacterium divergens	1	4.1	11.5	1	4.5	10.2	1	5.0	13.8
		Bacillus									
		Hafnia alvei	1	3.5	7.4	1	3.6	6.0	1	7.7	14.1
		Hafnia alvei	1	3.7	7.4	1	3.6	6.6	1	7.0	16.0
		Hafnia alvei	1	3.6	7.9	1	3.6	8.9	1	4.5	11.3
		Pseudomonas		65.4	13.8	1	9.7	19.1			
		Serratia	1	4.5	10.2	1	5.0	9.2	1	8.6	14.7
		Carnobacterium maltaromaticum	1	6.5	16.7	1	6.7	12.5	1	9.1	16.1
		Serratia	1	4.4	11.1	1	5.0	8.6	1	8.2	14.5
		% showing growth or average	**8**	**11.2**	**10.8**	**9**	**5.2**	**10.1**	**8**	**7.1**	**14.2**

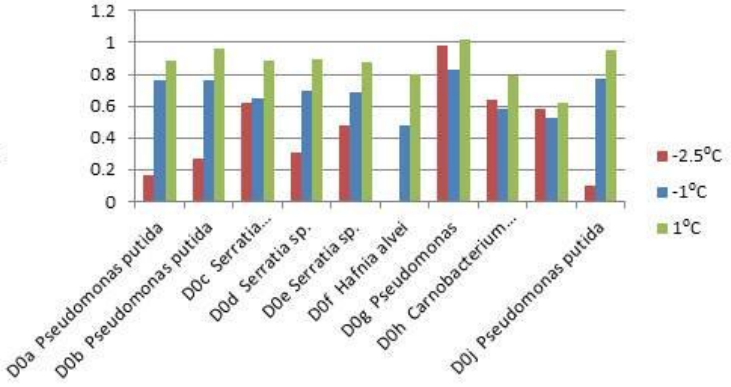
		Carnobacterium maltaromaticum				1	6.6	11.0	1	8.2	15.3
		Carnobacterium maltaromaticum	1	3.7	13.1	1	4.6	11.3	1	8.8	13.0
		Staphylococcus epidermidis	1	7.3	14.2						
		Carnobacterium maltaromaticum	1	10.5	15.0						
		Leuconostoc sp.									
F	30	Leuconostoc sp.									
		Leuconostoc sp.									
		Leuconostoc carnosum									
		Carnobacterium maltaromaticum	1	5.6	15.4	1	5.9	11.3	1	18.8	11.6
		Leuconostoc carnosum									
		% showing growth or average	**4**	**6.8**	**14.4**	**3**	**5.7**	**11.2**	**3**	**11.9**	**13.3**

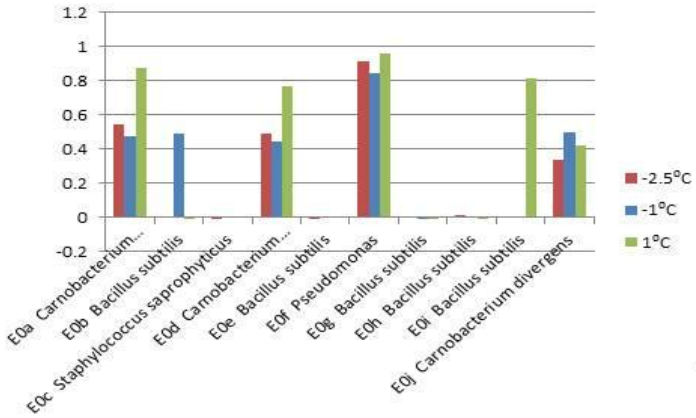
Appendix 4. Growth (OD_{540}) of week-1 isolates at -2.5, -1.0 and 1.0°C in BHI broth at 5 weeks.

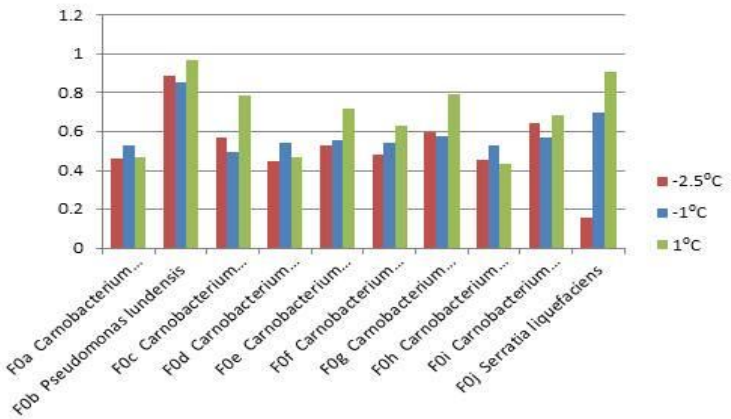




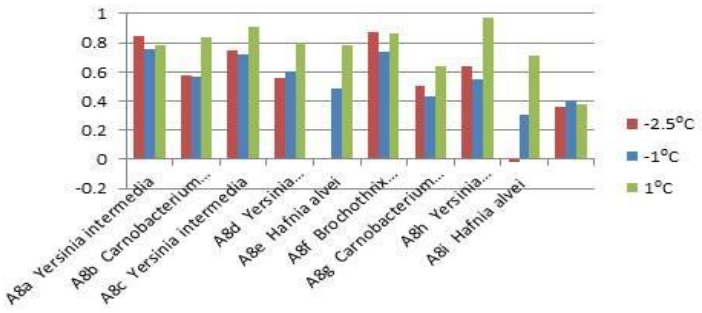


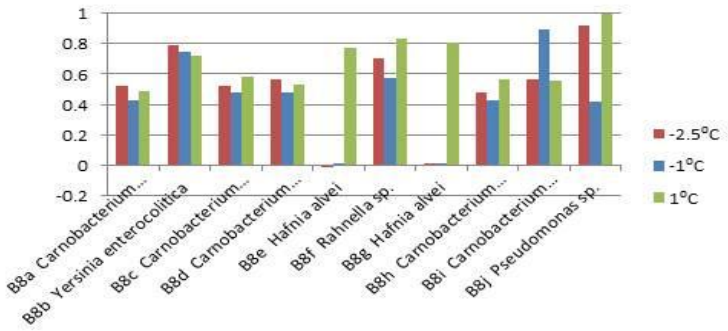


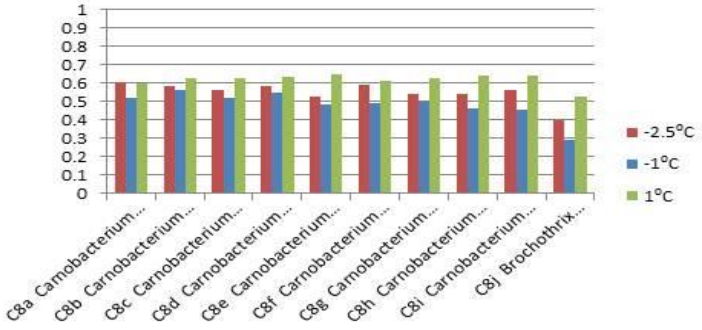


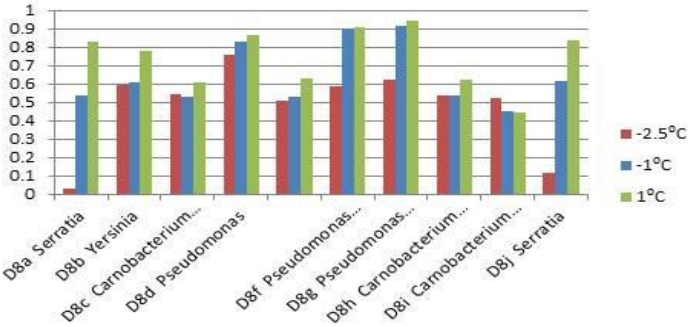


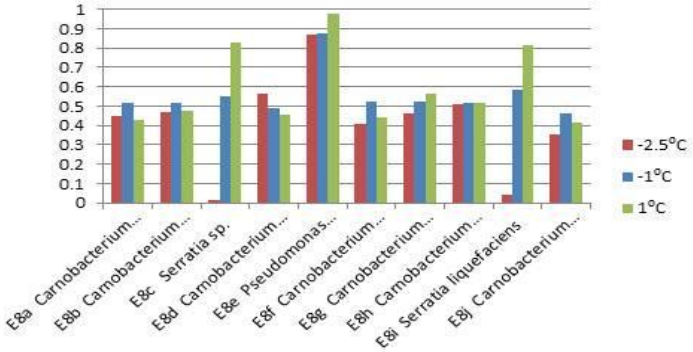
Appendix 5. Growth (OD_{540}) of week-8 isolates at -2.5, -1.0 and 1.0°C in BHI broth at 5 weeks.

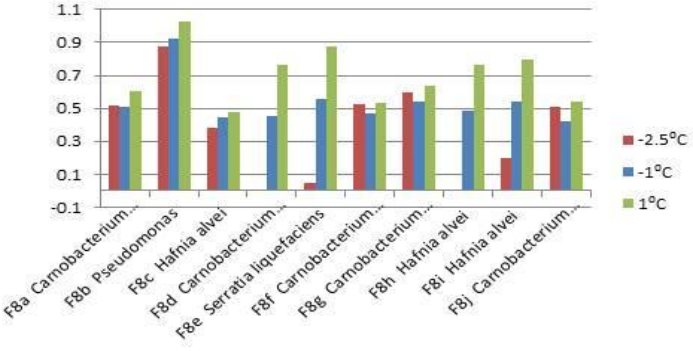




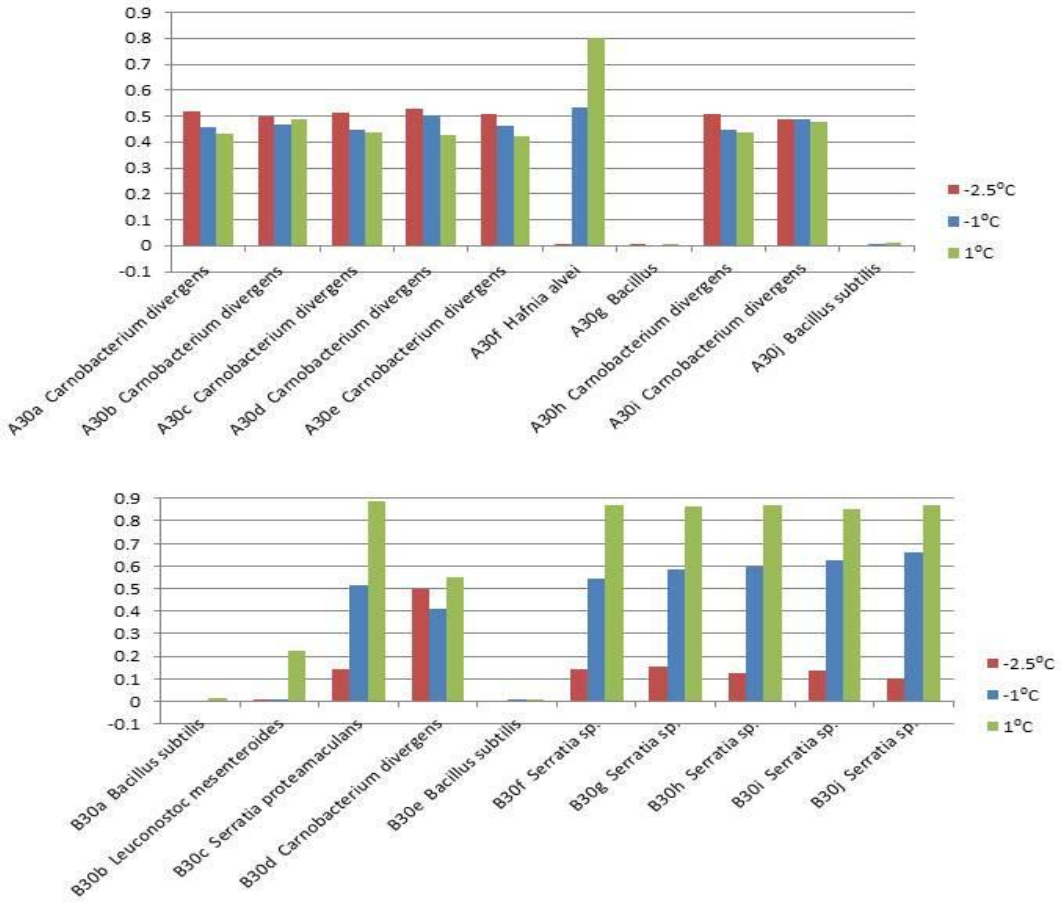




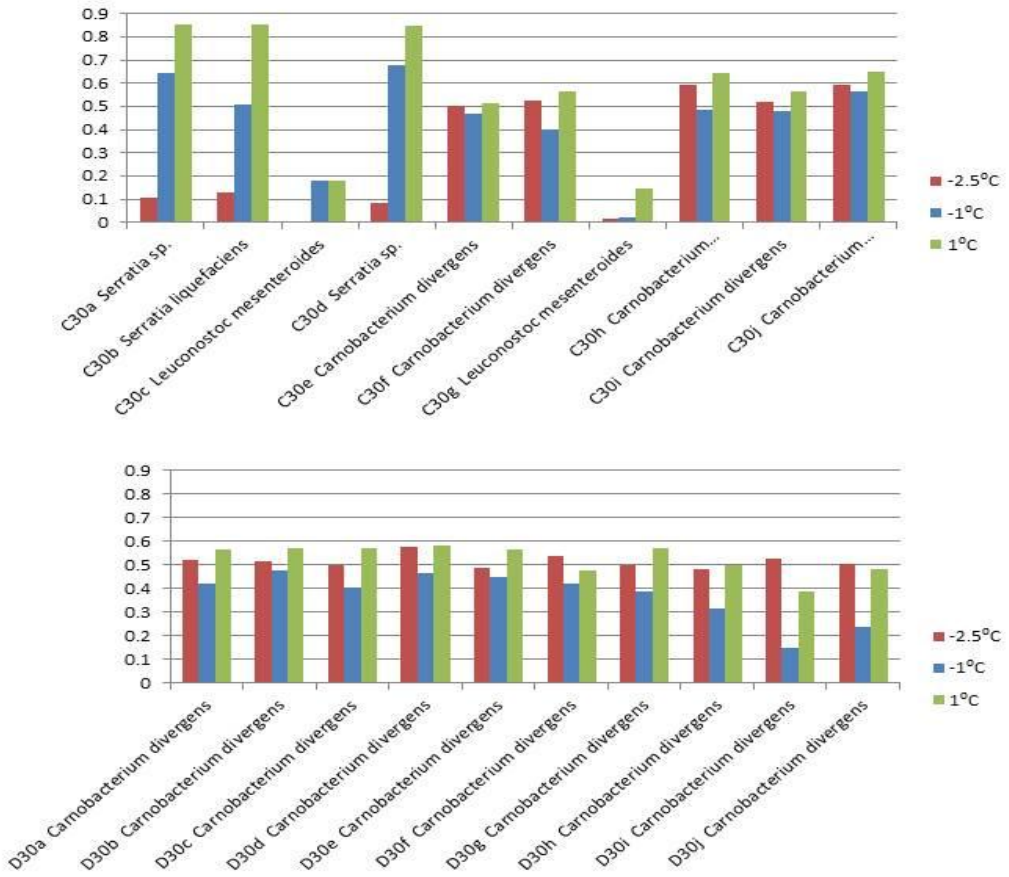




Appendix 6. Growth (OD_{540}) of week-30 isolates at -2.5, -1.0 and 1.0°C in BHI broth at 5 weeks.



Appendix 6 (cont). Growth (OD_{540}) of week-30 isolates at -2.5, -1.0 and 1.0°C in BHI broth at 5 weeks.



Appendix 6 (cont). Growth (OD_{540}) of week-30 isolates at -2.5, -1.0 and 1.0°C in BHI broth at 5 weeks.