

# The potential of lactic acid bacteria as biopreservatives of fresh meat

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## A range of bacteria contaminate meat during production.

- Meat processing typically results in the contamination of products from low numbers of a wide range of bacteria acquired from environmental sources such as water, fleece/hide and faeces. These organisms can grow during storage leading to potential spoilage and disease risks.
- The post processing interventions of vacuum-packaging and chilling restricts the range of species able to grow to those cold tolerant anaerobes able to utilise the narrow range of carbon sources available on the relatively low pH meat surface. These include members of the 'lactic-acid bacteria' (LAB) group of organisms (e.g. *Lactobacillus* and *Leuconostoc*) as well as cold tolerant spoilers such as *Brochothrix thermosphacta* and *Clostridium estertheticum*.
- However, although chilled storage conditions are sufficient to curtail the growth of mesophilic organisms, such as *Campylobacter* spp. and *E. coli*, many of these species remain viable during storage and present a source of risk post-storage when meat temperature rises during further processing, retail storage and consumption.



*C. estertheticum* is an important spoilage agent in the New Zealand meat industry. It is able to grow under chilled-meat conditions and causes 'blown-pack' spoilage.  
Photo credit: Jackie Boerema, AgResearch

## Some contaminants are potential spoilers & pathogens .....

- A variety of spoilage organisms and potential pathogens are regularly isolated from New Zealand meat.

Serotype	Enterohaemolysin	Shiga-toxin	No. of isolates	Year of isolation
O5:H-	+	I	3	1998
O6:H-	+	II	1	1998
O8:H9	-	-	1	1997
O14:H14	-	-	1	1998
O75:H8	-	I	1	1998
O75:H8	+	I	2	2000
O75:H40	-	I	1	1998
O75:H40	+	III	1	1998
O75:H40	+	III	1	1999
O81:H26	+	I	2	1998
O91:H-	-	I	2	1997
O91:H-	-	III	1	1998
O91:H-	+	I	3	1997
O91:H-	+	I	1	1999
O91:H-	+	III	2	1999
O91:H49	+	I	1	1997
O104:H-	-	I	1	1998
O104:H7	-	I	1	1998
O104:H7	+	I	2	1998
O123:H10	+	I	1	1998
O128:H2	+	I	1	1998
O128:H2	+	I	1	1999
Ont:H-	-	I	1	1997
Ont:H-	+	I	1	1998
Ont:H11	+	I	2	1998
Ont:H16	-	I	1	1998
Ont:H19	-	I	1	1998
OR:H-	+	I	1	1998
OR:H-	+	I	1	1999
Total			40	

- For example, toxigenic strains of *E. coli* have been reported from sheep meat, although serotypes of concern to human health have yet to be regularly reported.

Interventions to reduce the survivability of *E. coli* on meat will reduce the risk to health and market access.

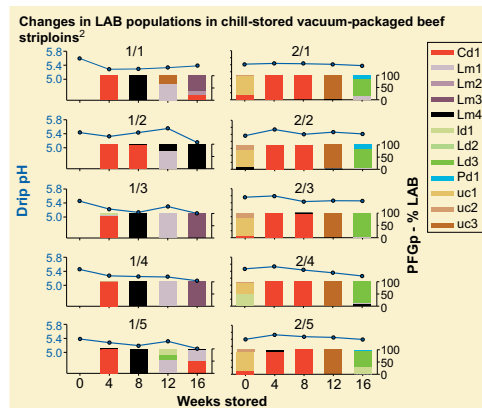
Serotypes and virulence factors of *E. coli* isolated from New Zealand sheep meat<sup>1</sup>

## ... whereas LAB contaminants have useful biopreservative properties ...

- The ability of LAB to grow on meat is potentially useful because of their biopreservative properties. They grow naturally on chilled meat, produce relatively little spoilage themselves, modify the environment to discourage the growth of other organisms, and can produce antimicrobial compounds such as bacteriocins.

## ... that could be enhanced if selected strains can be consistently grown on product.

- After several weeks of chilled storage (e.g. by the time product reaches European markets) selective and succession processes within the vacuum-pack ecosystem tend to drive a favoured few species of LAB to develop into dominant populations.
- However, different populations tend to develop on otherwise similar meat cuts - probably influenced by the random mix of contaminants that seed each cut.
- In the example, two sets of five replicate beef striploins (1/1-1/5 and 2/1-2/5) were stored for 16 weeks and were found to develop different populations of LAB (determined by comparing SmaI PFGE patterns).
- A seeding process that results in the consistent development of selected antimicrobial LAB strains would be useful for improving product uniformity, storage life, and reducing the risk of targeted spoilage organisms and pathogens surviving.



## We have attempted this with a strain of *Leuconostoc* ...

- Initial experiments were performed to see whether a strain of *Leuconostoc gasicomitatum* (Leu14), isolated earlier as the dominant LAB population on stored vacuum-packed chilled sheep meat after 12 weeks storage, could be used to establish significant populations on sheep meat after seeding fresh product with low numbers and storing.
- The results show how five packs of lamb shoulders (Packs 26-30), seeded with 100cfu Leu14 each, developed significant populations of the seed strain after 12 weeks storage.
- However, although these initial results with Leu14 were encouraging, leuconostocs can nevertheless spoil meat under certain processing situations so it might be prudent to also search for useful biopreservative strains amongst the more socially acceptable aciduric lactobacilli.

Development of *Leuconostoc gasicomitatum* (Leu14) populations on seeded stored vacuum-packaged chilled sheep meat<sup>3</sup>

Pack Treatment	Storage	pH meat	pH drip	Count (log)	Count (log)	Count (log)	Count (log)	Count (log)	Count (log)
1 10 cfu pack Leu14	12wk	5.8	6.2	6.1	3.6	5.4	0	3	2.4
2 10 cfu pack Leu14	12wk	5.8	6.2	6.1	2.7	5.4	0	2	2.8
3 10 cfu pack Leu14	12wk	5.6	6.1	6.1	2.6	5.7	0	3	2.8
4 10 cfu pack Leu14	12wk	6.1	6.2	6.5	2.1	5.6	0	3	2.9
5 10 cfu pack Leu14	12wk	5.9	6.3	6.4	3.2	5.4	0	1	2.8
6 10 cfu pack Leu14	12wk	avg. 5.8	6.2	6.2	2.8	5.5	2.4	2.7	2.6
7 10 cfu pack Leu14	12wk	6.5	6.7	7.5	5.4	5.9	0	5	
8 10 cfu pack Leu14	12wk	6.3	6.4	7.5	5.5	6.9	0	4.5	
9 10 cfu pack Leu14	12wk	6.1	6.2	7.7	4.2	7.4	0	3.5	
10 10 cfu pack Leu14	12wk	5.7	6.1	7.3	4.1	4.5	0	4	
11 10 cfu pack Leu14	12wk	5.8	5.9	6.8	4.0	6.1	0	3	
12 10 cfu pack Leu14	12wk	avg. 6.1	6.2	7.4	4.6	6.2	4.0		
13 10 cfu pack Leu14	12wk	6.1	6.2	6.2	2.5	5.4	0	2	2.9
14 10 cfu pack Leu14	12wk	5.2	6.2	5.9	2.2	5.2	0	2	3.8
15 10 cfu pack Leu14	12wk	5.8	6.3	6.1	2.3	5.6	0	2	2.9
16 10 cfu pack Leu14	12wk	6.0	6.5	5.8	2.2	5.2	0	2	3.1
17 10 cfu pack Leu14	12wk	5.2	6.0	5.6	2.1	4.6	0.20	3	2.6
18 10 cfu pack Leu14	12wk	5.9	6.2	5.9	2.2	5.2	2.2	3.0	2.8
19 10 cfu pack Leu14	12wk	5.8	5.7	6.7	4.2	5.7	0.80	3.5	
20 10 cfu pack Leu14	12wk	5.5	6.1	6.9	4.1	6.5	0.80	2	
21 10 cfu pack Leu14	12wk	6.0	5.9	7.4	4.0	5.9	0	5	
22 10 cfu pack Leu14	12wk	5.7	5.7	8.6	3.8	5.3	0	4.5	
23 10 cfu pack Leu14	12wk	5.7	6.1	7.1	4.4	6.5	0.25	5	
24 10 cfu pack Leu14	12wk	avg. 5.9	5.9	7.3	4.1	5.9	4.0		
25 10 cfu pack Leu14	12wk	5.9	6.2	5.6	0.1	2.1	1.00	2	3.0
26 10 cfu pack Leu14	12wk	5.2	6.0	6.0	1.4	5.1	0.05	4	2.4
27 10 cfu pack Leu14	12wk	5.1	5.9	5.9	1.3	4.3	0.05	1	2.5
28 10 cfu pack Leu14	12wk	5.3	6.2	6.0	3.4	5.2	0.05	2	3.0
29 10 cfu pack Leu14	12wk	5.3	6.2	6.5	2.9	5.5	0	3	2.9
30 10 cfu pack Leu14	12wk	5.4	6.1	6.5	1.8	4.4	2.4	2.8	2.4
31 10 cfu pack Leu14	12wk	6.3	6.2	7.3	4.5	4.0	1.00	5	
32 10 cfu pack Leu14	12wk	6.0	6.2	6.9	4.7	5.3	0.70	5	
33 10 cfu pack Leu14	12wk	5.9	5.9	9.2	4.0	7.8	0.95	3	
34 10 cfu pack Leu14	12wk	6.0	6.1	8.9	4.6	6.5	1.00	5	
35 10 cfu pack Leu14	12wk	6.0	6.2	8.5	5.1	4.8	0.50	5	
36 10 cfu pack Leu14	12wk	6.0	6.1	8.1	4.6	5.5	4.6		
37 10 cfu pack Leu14	12wk	5.6	6.1	4.6	2.6	3.9	0	1	3.7
38 10 cfu pack Leu14	12wk	5.8	6.3	5.4	1.5	4.2	0	2	2.8
39 10 cfu pack Leu14	12wk	6.0	6.1	3.7	1.7	2.8	0	4	2.3
40 10 cfu pack Leu14	12wk	5.8	6.3	4.0	1.5	3.8	0	2	2.7
41 10 cfu pack Leu14	12wk	6.0	6.1	6.0	4.4	4.9	0	3	
42 10 cfu pack Leu14	12wk	5.8	6.1	7.8	4.8	6.7	3.9		

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## ... and are now looking at lactobacilli for their potential as biopreservatives.

- Lactobacillus sakei* is a potentially valuable biopreservative strain because it is relatively innocuous, has a long history as a favoured food starter, and produces compounds that restrict the growth of unwanted organisms.<sup>4</sup>
- INRA has shown that *L. sakei* possesses genetic factors related to survival and performance which are potentially exploitable for enhancing this organism's competitiveness under storage conditions.<sup>5, 6, 7</sup>

*L. sakei* isolated from salami.  
Photo credit: Anika Marceau, INRA



## Future work

- A three-year collaboration has started between AgResearch, INRA and the Universities of Otago and Paris to explore the biopreservative properties of LABs isolated from New Zealand meat.
- The study will focus predominantly on *L. sakei* and many of the strains under study were isolated during contract work for Meat and Wool New Zealand.
- Combining the relative strengths of each group will result in the identification and better understanding of strains that will be later evaluated in commercial storage trials.

## In particular, the present study will evaluate:

- generation and nature of molecules antagonistic to unsavoury organisms (e.g. toxigenic *E. coli* and *Campylobacter*)
- ability to compete for and utilise carbon sources (e.g. co-metabolism of glucose and ribose)
- survival mechanisms at chill temperatures (e.g. possession of genes relating to cold adaptation)
- Ultimately, this programme assists in the evaluation of biopreservation as a useful technology.