DANISH TECHNOLOGICAL INSTITUTE

Note

Salt reduction in fermented sausages and bacon

Challengetest on bacon with a reduced salt content

27 March 2023
Project No. 2010421
Version 1
Init. NBS/MT/AGLK

Background

Reduction of the salt content in processed food including meat products has a high focus among health authorities, food producers and the consumers. Reduction of the salt content may impact food safety; however, it is possible to predict growth of many pathogenic bacteria by using predictive models. But if the salt concentration becomes very low, using predictive models can become difficult. Likewise, the models' content of preservative variables may be limited in relation to the industrial wishes for preservation profile.

An initial assessment of the food safety impacts of reducing the salt content in bacon concluded that more knowledge was needed regarding growth of *Bacillus cereus*, *Yersinia enterocolitica* and *Salmonella* in bacon with a reduced salt content that is stabilised against growth of *Listeria monocytogenes*.

Aim

The aim of the experiment is to examine if there is a risk of growth of *Salmonella*, *Bacillus cereus* and *Yersinia enterocolitica* in bacon with a reduced salt content that is stabilised against growth of *Listeria monocytogenes* during storage at 5-7°C for a minimum of 56 days.

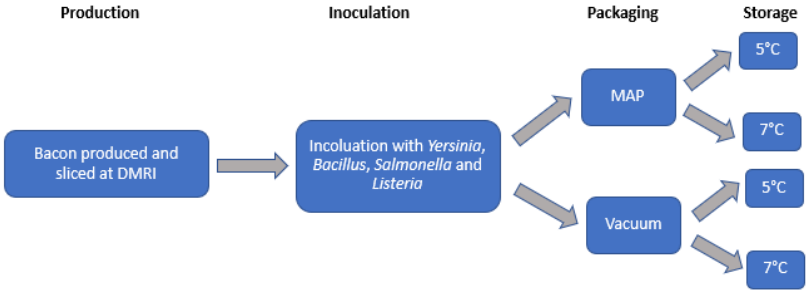
The hypothesis is that if a product is stabilised against growth of *Listeria monocytogenes*, no other pathogens will grow in the product.

Conclusion

- *Listeria monocytogenes* did not grow in the two bacon products with a low salt content that according to predictive microbiology were stabilised against growth of Listeria.
- The experimental data support the hypothesis that *Salmonella*, *Yersinia enterocolitica* and *Bacillus cereus* do not grow in a product that is stabilised against growth of *Listeria monocytogenes*.
- In particular *Yersinia enterocolitica* was impaired by addition of organic acids, but also *Salmonella* is affected by the addition of organic acids.
- In bacon with a normal salt content (2.4%), *Listeria* increased to 7 log cfu/g during 11 weeks of storage at 5 or 7°C. Of the other pathogens, only *Yersinia* increased in this product and only when packed in vacuum packs while *Salmonella* and *Bacillus cereus* either remained at the initial level or were reduced during the storage period.

Method

Experimental overview



Variants of bacon included in the experiment:

1) Standard product (reference) (approx. 2.6-2.8% salt in product + nitrite (60 ppm))
2) LM-stabilised (+ nitrite (60 ppm), + lactate (2.18%)/acetate (0.17%)); approx. 2.1% salt in product
3) Bacon containing 1.3% NaCl in product (+ nitrite (60 ppm), + lactate (2.18%)/acetate (0.17%))

Bacon production

Recipes for the three brines are included in Appendix 1.

Pork belly was brine injected (55 beats per min.; pressure of 1.6 bar) and tumbled (30 min; 6 rpm).

Smoking was performed according to the following program:

Process	Temp.	Time
Drying	60°C	20 min
Smoke	60°C	25 min
Drying	60°C	3 min
Smoke	60°C	20 min
Ventilation	60°C	10 min

The bacon sides were vacuum packed and stored frozen. Before slicing, they were tempered to -5-7°C.

The bacon sides were sliced into 2 mm thickness.

Pathogenic bacteria

The following strains of pathogenic bacteria from DMRI's strain collection were included:

Salmonella:

- DMRICC 4983-PX
- DMRICC 4984-PX
- DMRICC 4985

Y. enterocolitica:

- DMRICC 4297-PX – biotype 4, serotype O:3
- DMRICC 4314-PX – biotype 2 or 3, serotype O:9
- DMRICC 5021-PX – biotype 2, serotype O:9

B. cereus:

- *B. cereus* DMRICC 4826-PX (MC118)
- *B. weihenstephanensis/cereus* MC67 DMRICC 3880-PX
- *B. cereus* DMRICC 3759-PX
- *B. cereus* DMRICC 4623-PX

Listeria monocytogenes:

- DMRICC 3012-PX
- DMRICC 4127-PX
- DMRICC 4140-PX

The strains were cultivated as described in Appendix 2.

Before the experiment, all strains were tested for cross reactions on all the selective media included in the analyses (Rapid'L.mono, CAY, SSI and BBCA).

Inoculation cocktail

All strains were mixed to one inoculation cocktail 1:1:1:1.

Inoculation

Slices of bacon were placed in plastic trays (1 slice per tray, approx. 20 g) and surface inoculated to a theoretical level of approx. 3 log cfu/cm^2 or 3 log cfu/g.

Packaging and storage

The trays were packed in vacuum bags with low oxygen permeability (PM Pack PA/PE 220; OTR = 12 $cm^3/m^2 \cdot d \cdot bar$) and packed either with modified atmosphere or vacuum.

The packages were stored at 5±1°C and 7±1°C.

Microbial plate counts

Plate counts were determined for the following selective substrates:

- *Salmonella:* SSI agar (37°C/1 day)
- *Yersinia enterocolitica:* (CHROMAgar Yersinia) (30°C/2 days)
- *Bacillus cereus:* Brilliance Bacillus cereus agar (Oxoid CM 1036) with suppl. 0230 (30°C/2-3 days)
- *Listeria monocytogenes:* Rapid'L.mono (37°C/1 day)

In addition, background flora was determined in non-inoculated packages on BHI (20°C/5 days).

Chemical analyses

The bacon slices were analysed for pH, water, salt, lactate, and acetate.

Gas composition was measured in MA-packs on day 0 and at the sampling points.

Results and discussion

Chemical analyses

Table 1. Results of chemical analyses.

Recipe	pH	Salt (NaCl) %	Water %	Salt/water % (calculated)*	Ace-tate	L-lac-tate
"Reference"	5.72	2.40	49.0	4.9	n.d.	n.d.
"2.1% salt"	5.80	2.05	51.9	3.9	0.17	1.76
"1.3% salt"	5.80	1.57	59.5	2.6	0.20	2.01

*: The results are subject to some uncertainty as the samples were inhomogeneous.

The salt level in the bacon with the lowest amount of salt added ("1.3% salt") was a little higher than the aimed concentration. Also, the concentration of acetate was slightly higher than the aimed concentration in this bacon whereas the concentration of lactate was a little lower than the aimed concentration in both of the two pieces of bacon with a reduced salt content.

Gas composition in MA-packs

Table 2. O_2 and CO_2 in MA-packs

	O_2 (%)	CO_2 (%)
Start	0.083	29.6
Week 4_5°C_1.6% salt	0.744	21.1
Week 4_5°C_1. 6% salt_background flora	0.675	21.9
Week 8_5°C_1.6% salt	1.199	18.3
Week 8_5°C_1.6% salt_background flora	0.632	24.7
Week 11_5°C_1.6% salt	1.649	15.3
Week 11_5°C_1.6% salt_background flora	1.685	15.1
Week 4_7°C_1.6% salt	0.333	26.2
Week 4_7°C_1.6% salt_background flora	0.317	26.5
Week 8_7°C_1.6% salt	0.586	25.1
Week 8_7°C_1.6% salt_background flora	1.143	18.7
Week 11_7°C_1.6% salt	0.814	23.8
Week 11_7°C_1.6% salt_background flora	0.807	23.7
Week 11_5°C_2.1% salt	1.797	14.9
Week 11_5°C_2.1% salt_background flora	1.605	15.9
Week 11_7°C_2.1% salt	0.839	23.7
Week 11_7°C_2.1% salt_background flora	0.773	24.1
Week 11_5°C_ref	1.573	15.8
Week 11_5°C_ref_background flora	1.628	16.1
Week 11_7°C_ref	0.594	23.9
Week 11_7°C_ref_backgorund flora	0.746	24

Table 2 shows that CO_2 diffused out of the MA-packs during storage, whereas O_2 diffused into the packs.

During storage, the concentration of CO_2 in MA-packs at 7°C was higher than at 5°C, indicating that more CO_2 was produced microbially under storage at 7°C than at 5°C.

At 5°C, the concentration of O_2 and CO_2 were comparable for the three types of bacon after 11 weeks of storage, indicating no or minimal difference in microbial growth between the three types of bacon.

At 7°C, the concentration of O_2 was higher in the reference than in the two pieces of bacon with a reduced salt content, indicating more growth in the reference than in the bacon with a reduced salt content.

Growth or reduction of bacteria in bacon with a normal salt level (2.4% salt in product)

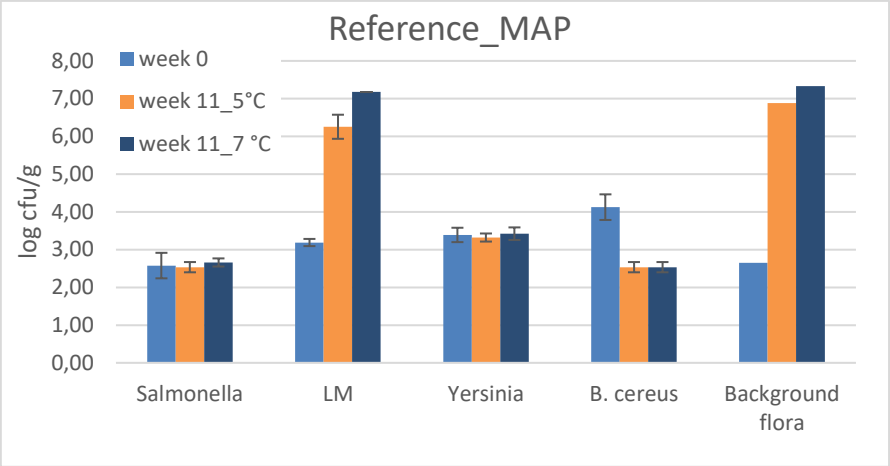


Figure 1. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in MA-packed bacon containing 2.4% salt in the product (4.9% salt in water). The limit of detection is 1 log cfu/g.

In MA-packed reference bacon (normal salt level), 3-4 log cfu/g growth of *Listeria monocytogenes* was observed within 11 weeks of storage at either 5°C or 7°C reaching 6-7 log cfu/g after 11 weeks of storage. The other three pathogens either remained stable at the inoculation level or declined (*Bacillus cereus*) during the storage period (Figure 1).

The background flora increased in numbers to 7 log cfu/g within the 11 weeks of storage.

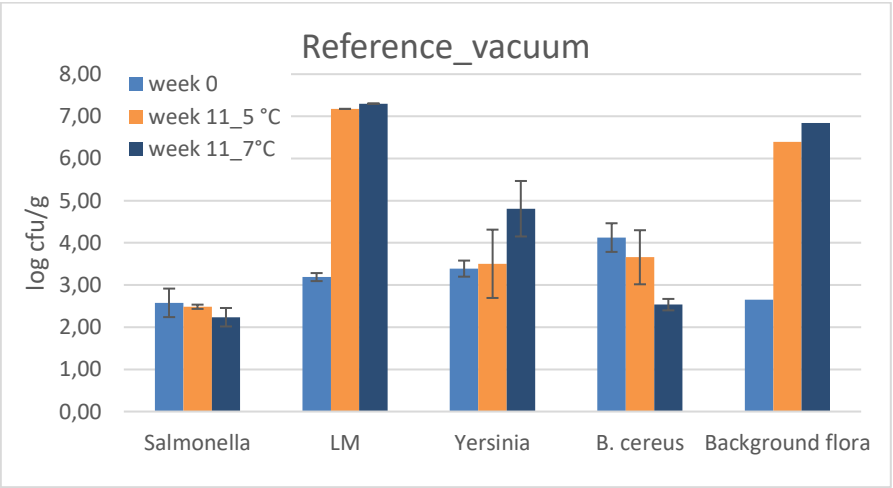


Figure 2. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in vacuum-packed bacon containing 2.4% salt in the product (4.9% salt in water). The limit of detection is 1 log cfu/g.

Also, in vacuum-packed bacon with a normal salt level, *Listeria monocytogenes* increased in numbers to 7 log cfu/g within 11 weeks at either 5°C or 7°C (Figure 2). *Salmonella* did not grow, and as in MA-packs the number of *Bacillus cereus* declined during storage. *Yersinia enterocolitica* did not grow at 5°C, but did grow (approx. 1.5 log cfu/g) at 7°C. The data indicate that *Yersinia* grows better in bacon packed in vacuum than in bacon packed in MA (Figure 1 vs. Figure 2).

Growth or reduction in bacon with a reduced salt content (2.05% salt in product)

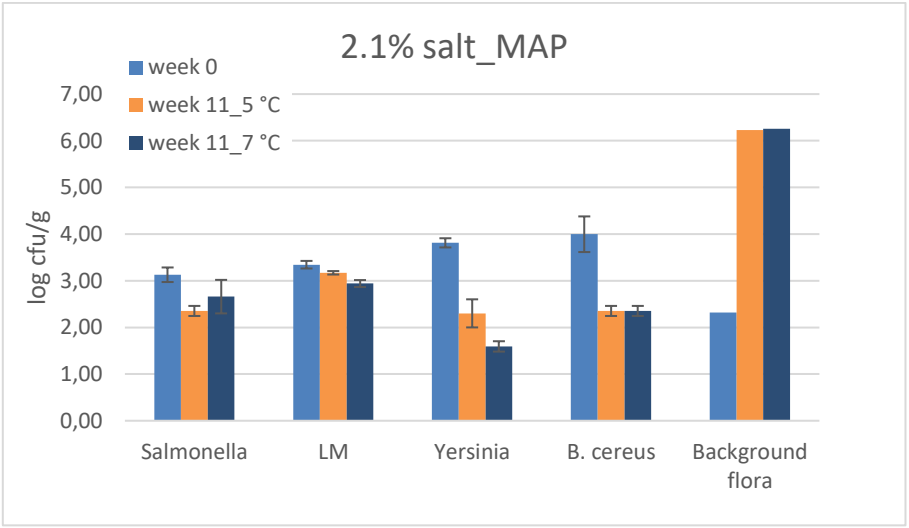


Figure 3. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in MA-packed bacon containing 2.1% salt in the product (3.9% salt in water), and lactate and acetate. The limit of detection is 1 log cfu/g.

When the salt content was reduced to 2.05% salt in the product, and lactate and acetate were added to stabilise against growth of *Listeria monocytogenes*, no growth of any of the pathogens was observed with 11 weeks of storage at either 5°C or 7°C in MA-packs (Figure 2). Both *Yersinia enterocolitica* and *Bacillus cereus* were reduced in numbers (reduction of approx. 1.7-2.2 log cfu/g) during the storage period at both 5°C and 7°C.

The background flora increased from 2.3 log cfu/g to 6 log cfu/g within 11 weeks, which was lower compared to 7 log cfu/g in the reference bacon with a normal salt content (Figure 1). This indicates that the preservation with a lower content of salt + organic acids provides a better inhibition than the standard salt/nitrite preservation.

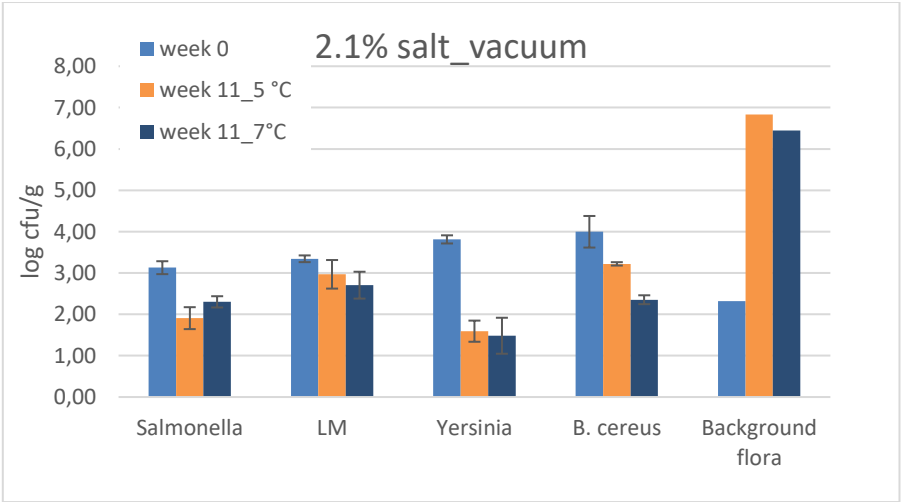


Figure 4. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in vacuum-packed bacon containing 2.1% salt in the product (3.9% salt in water), and lactate and acetate. The limit of detection is 1 log cfu/g.

As in the MA-packs, no growth of pathogens was observed in the vacuum-packed bacon with a reduced salt content (Figure 4). On the contrary, for *Salmonella, Yersinia* and *Bacillus* the reduction was observed at both storage temperatures.

The background flora grew to approx. 6 log cfu/g.

Growth or reduction in bacon with a reduced salt content (1.6% salt in product)

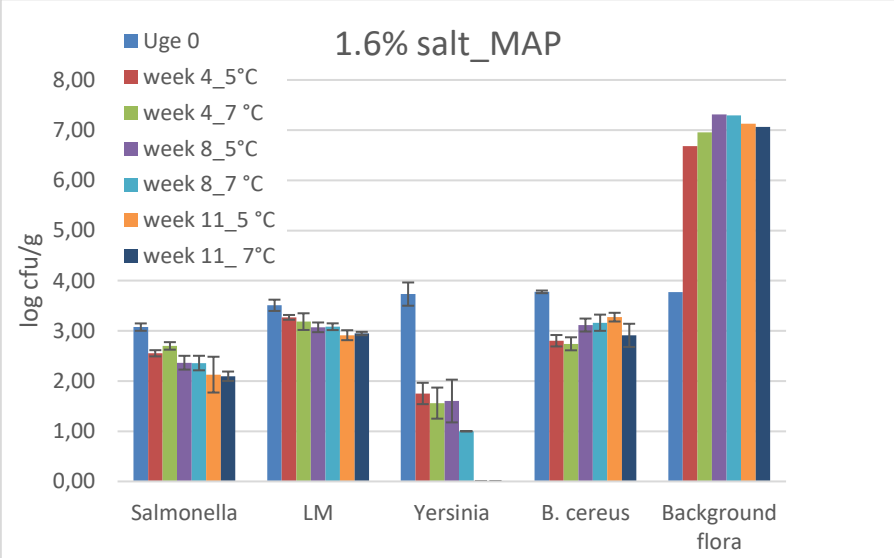


Figure 5. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in MA-packed bacon containing 1.6% salt in the product (2.6% salt in water), and lactate and acetate. The limit of detection is 1 log cfu/g.

Also, in MA-packed bacon with a highly reduced salt content of 1.6% salt in the product, no growth of any of the pathogens was observed within 11 weeks of storage at either 5°C or 7°C (Figure 5).

Both *Salmonella*, *Yersinia enterocolitica*, and *Bacillus cereus* were reduced in numbers (reduction of approx. 1-2.2 log cfu/g) during the storage period at both 5°C and 7°C.

Yersinia was reduced more in bacon containing 1.6% salt compared to the bacon containing 2.1% salt. The bacon containing 1.6% of salt had a slightly higher concentration of both lactate and acetate (Table 1), which could indicate that the presence of organic acids has a higher impact on growth of *Yersinia* than the concentration of salt (NB! a_w was not measured).

The background flora increased from 3.8 log cfu/g to approx. 7 log cfu/g within 4 weeks of storage. It is likely that the background flora contributes to inhibition of growth of the pathogenic bacteria.

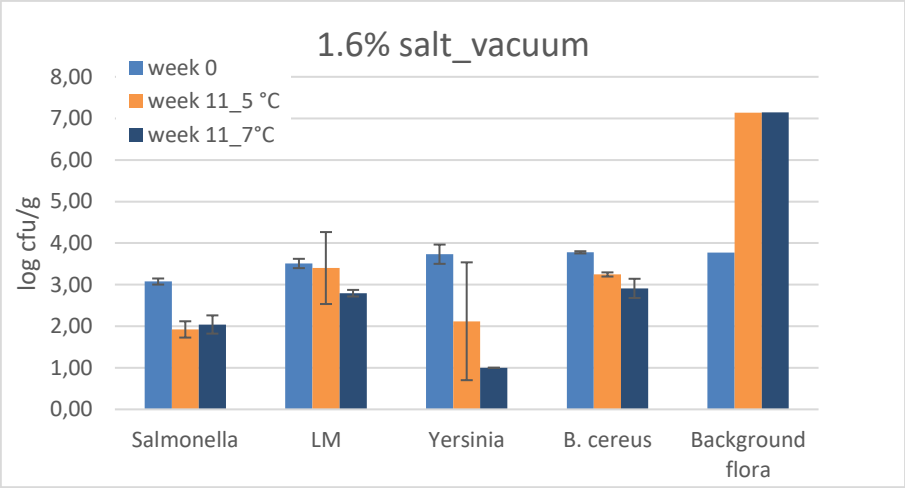


Figure 6. Growth or reduction of *Salmonella*, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Bacillus cereus* in vacuum-packed bacon containing 1.6% salt in the product (2.6% salt in water), and lactate and acetate. The limit of detection is 1 log cfu/g.

The pattern of pathogen reduction in the vacuum-packed bacon with a highly reduced salt content was comparable to the MA-packed bacon with a highly reduced salt content (Figure 6). It is not known how fast the background flora reached 7 log cfu/g. It might have been after 4 weeks as seen for the MA-packed bacon.

Conclusion

- *Listeria monocytogenes* did not grow in the two bacon products with a low salt content that according to predictive microbiology were stabilised against growth of Listeria.

- The experimental data support the hypothesis that *Salmonella*, *Yersinia enterocolitica* and *Bacillus cereus* do not grow in a product that is stabilised against growth of *Listeria monocytogenes*.

- In particular *Yersinia enterocolitica* was impaired by addition of organic acids, but also *Salmonella* is affected by the addition of organic acids.

- In bacon with a normal salt content (2.4%), *Listeria* increased to 7 log cfu/g during 11 weeks of storage at 5 or 7°C. Of the other pathogens, only *Yersinia* increased in this product and only when packed in vacuum packs while *Salmonella* and *Bacillus cereus* either remained at the initial level or were reduced during the storage period.

Recipes

Recipe 1: Bacon with **2.8%** salt, 60 ppm nitrite. 15% growth and 5% loss when smoking.

Brine composition
Nitrite salt 7.3%
Vacuum salt 13.2%
Ascorbate 0.02%
Water 79.5%

	10 kg brine	50 kg brine
Water	7.95 kg	39.75 kg
Red nitrite salt (0.6%)	0.73 kg	3.65 kg
Vacuum salt	1.32 kg	6.60 kg
Na-ascorbate	0.002 kg	0.01 kg

Recipe 2: Bacon with **2.1% salt**, 60 ppm nitrite, 2.18% lactate and 0.17% acetate. 15% growth and 5% loss when smoking.

Brine composition
Nitrite salt 7.3%
Vacuum salt 8.1%
Na-lactate (Purasal 60% solution) 26.6%
Na-acetate (powder 100%) 1.3%
Na-ascorbate 0.02%
Water 56.7%

	10 kg brine	50 kg brine
Water	5.67 kg	28.35 kg
Red nitrite salt (0.6%)	0.73 kg	3.65 kg
Vacuum salt	0.81 kg	4.05 kg
Na-acetate (powder)	0.13 kg	0.65 kg
Na-lactate (Purasal 60% solution)	2.66 kg	13.30 kg
Na-ascorbate	0.002 kg	0.01 kg

Recipe 3: Bacon with **1.3% salt**, 60 ppm nitrite, 2.18% lactate and 0.17% acetate. 15% growth and 5% loss when smoking.

Brine composition
Nitrite salt 7.3%
Vacuum salt 2.2%
Na-lactate (Purasal 60% solution) 26.6%
Na-acetate (powder 100%) 1.3%
Na-ascorbate 0.02%
Water 62.6%

	10 kg brine	50 kg brine
Water	6.26 kg	31.30 kg
Red nitrite salt (0.6%)	0.73 kg	3.65 kg
Vacuum salt	0.22 kg	1.10 kg
Na-acetate (powder)	0.13 kg	0.65 kg
Na-lactate (Purasal 60% solution)	2.66 kg	13.30 kg
Na-ascorbate	0.002 kg	0.01 kg

Bacterial cultivation

Salmonella

The strains were recovered from DMRI's collection (-80°C) and purified on BHI agar.

After standard purity control, they were cultured in BHI broth at 30°C for 3 days (over the weekend) (inoculation with inoculation needle from a single colony; the final germ count was approx. 10^9 CFU/ml).

The three cultures were mixed (1:1:1) and diluted 100X. The germ count was determined on BHI agar (expected germ count approx. 10^7 CFU/ml).

Yersinia enterocolitica

The strains were taken from DMRI's collection (-80°C) and awakened in BHI broth at 30°C overnight (ON). The cultures were streaked onto BHI agar plates and CAY agar. The plates were incubated at 30°C ON. BHI-A and CAY were checked for purity and uniform colonies. BHI-A was stored in a refrigerator, and from this a loop-full colony mass was taken with an eye inoculum needle and inoculated into 10 ml of BHI-B and incubated at 37°C for 1 day. Expected final ~~spg~~erm count approx. 10^{8-9} cfu/ml.

B. cereus

The strains were awakened from freezing and purified on BHI agar. From a well-isolated colony, a small amount of material was transferred with a pointed inoculation needle to 10 ml of BHI at 30°C for 1 day aerobically.

0.1 ml of the BHI overnight culture was transferred to the sporulation medium (T-3), and the agar plates were incubated at 30°C for 6 days. The spores were harvested from the sporulation medium with a sterile cotton swab and suspended in 10 ml of ice-cold sterile FK. The spores were centrifuged in a cooled centrifuge for 10 min. at 5000 rpm and resuspended in 10 ml of ice-cold sterile FK. Expected spore count was 10^7 per ml. The 4 cultures were mixed to a cocktail (1:1:1:1), which was diluted to -1 with ice-cold sterile FK.

T-3 agar

Preparation of T-3 agar (1 litre):
3 g of tryptone
2 g tryptose
1.5 g of yeast extract
6.9 g of sodium dihydrogen phosphate with 1xH_20
0.008 g of $MnCl_2$ with 4xH_20
15 g of agar

The substances were dissolved except agar in e.g., 900 ml of water, the pH was adjusted.

Agar was added and filled up to 1 l.

The pH was adjusted with NaOH to 6.8.

Reference: Travers et al. (1987), AEM, pp. 1263-1266.

Autoclaved at 121°C for 10 min.

L. monocytogenes

The strains were recovered from DMRI's collection (-80°C) and purified on BHI agar.

After standard purity control, they were cultured from BHI-A up into BHI-B at 30°C for 3 days (over the weekend) (inoculation with inoculation needle from a single colony; the final germ count was approx. 10^9 CFU/ml).

The 3 cultures were mixed (1:1:1) and diluted 100X, the germ count was determined on BHI-A (expected germ count approx. 10^7 CFU/ml).