Acid treatment for improved recovery of STEC from enrichment broths

Gro S. Johannessen
Section for food bacteriology
Norwegian Veterinary Institute, Oslo, Norway

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Background and introduction

- Current ISO/TS 13136:2012 is based on screening with PCR for specific genes followed by attempts of isolation
- Isolation from PCR positive samples – picking of 50 colonies
- Low isolation rate from PCR positive samples – challenge for food industry, competent authorities and research
- An isolate is still needed for most characterization purposes, comparisons and tracing of contamination
- Significance and consequence of detection of a gene?

Some examples (from our lab)

- Meat imported from 3rd countries, project

	Bovine	Ovine
No. consignments	177	13
Total *stx* positive	42	12
Total *eae* positive	27	9
Stx and *eae* positive	10	8
Positive for serotype*	6	8
Only *stx* positive	32	4
Only *eae* positive	17	1
eae and O26 positive	6	0

**stx* and *eae* positive samples also PCR positive for O26, O103, O111, O145 or O157

Meat type	*E. coli*	Virulence factors	Comments
Bovine	O26	stx-, eae+	
Bovine	O26	stx-, eae+	Two samples from same consignment
Bovine	O26	stx-, eae+	
Bovine	O26	stx-, eae+	
Bovine	O26	stx-, eae+	
Mutton/lamb	O103	stx1+, eae+	
Mutton/lamb	O103	stx-, eae+	

Poster presented av VTEC 2015.

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Veg-i-Trade project

- Samples analysed with GeneDisc and ISO method
- 64 samples positive for *stx*/*eae* and one or more serotypes
- 9 samples were culture confirmed, isolation rate 14%
- STEC O26, O103, O111 and O157 were confirmed by cultures
- Poster presented at VTEC 2015

	No. of samples	Pres. pos. by PCR	Culture confirmed
Leafy greens	277	0	-
Water leafy greens	222	12	2
Soil leafy greens	371	28	3
Strawberries	152	0	-
Water strawberries	94	21	2
Soil/substrate strawberries	128	3	2

Sample type	N*	stx pos	eae pos	stx+eae pos	stx+eae+ serotype pos	Culture confirmed
Leafy greens	189	3	7	0	0	-
Soil leafy greens	256	25	91	23	23	0
Water leafy greens	101	9	19	6	6	0
Strawberries	80	0	6	0	0	-
Soil strawberries	80	1	48	1	1	0
Water strawberries	16	10	13	10	10	0

* Detailed results from two partners

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Challenge

How to increase the recovery of isolates from PCR positive enrichments?

Or how to look for a needle in the haystack when the needle looks like the straw……..

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Potential ways of increasing recovery

- Phenotypical traits
  - Different selective enrichments and plating media – strain variety
  - Plating of dilutions
  - Acid treatment – we started the work because of revision of *Shigella* methods
- Immunological approaches
  - (A)IMS – immunomagnetic separation – beads for a limited number of serotypes
  - Immunoblot
- DNA hybridization

Why acid treatment?

- *E. coli* – effective in resisting acid stress, may survive pH 2 for hours, depending on growth phase and growth medium
  - 3 acid resistance systems (AR1, 2 and 3) – collectively protect strains from different acid stress conditions in different environments
  - Strain variations
  - Link between Stx phage carriage and *E. coli* acid resistance? (Veses-Garcia et al, 2015)
- Reduce background

References: Foster (2004), Nature Reviews, November 2004, 2, 898-907; Kim et al. (2015) Journal of Food Science, 80 (10), 2257-2264; Large et al. (2005) AEM, 71 (5), 2493-2500; Veses-Garcia et al. (2015), AEM, doi:1128/AEM.02034-15,

Different approaches

- Different ways of doing acidification, but all includes treatment at pH2-3 for a variable period of time
  - Acidification of produce rinsate prior to enrichment
  - Exposure of enrichment broth to acid followed by plating (with or without a centrifugation step and with or without further enrichment)
  - Acid treatments of beads after IMS

References: Grant (2004), AEM, 70 (2), 1226-1230; Grant (2008), JFP, 71 (1), 139-145; Fedio et al (2012), Food Micro, 30, 83-90; Yoshimito et al (2012) J Food Sci, 77(8), 481-489; Fukushima & Goyomoda (1999), Zentralbl Bakteriol, 289(4), 415-428.

Acidification after IMS

- Treat bead-bacteria suspension with acid
  - Variations over a theme
  - Incubation @room temperature with or without shaking for 1hr
  - Dilute the sample (increase pH again)
  - Plate on usual plates (Rainbow, ChromAgar STEC and others depending on serotype)

Thanks to Catarina (NRL Sweden) and Saija (NRL Finland) for information☺

Acid treatment – two options from literature tested in our lab

Centrifugation and direct plating

Enrichment

Centrifuge 1 ml, 12000 x g, 3 min

↓

Resuspend in TSB (pH2), incubate 30 min, room temp

↓

Centrifuge 12000 x g, 3 min, resuspend in TSBYE, plating

Fedio *et al*. Food Microbiol, 2012, 83-90

Acid treatment and ON incubation

Enrichment in TSBYE

↓

Acid treatment

(1 ml in 9 ml TSB, pH 2, 30 min, room temp

↓

1 ml acidified enrichment to 9 ml TSBYE, 37°C, 24 hrs, plating

Grant , AEM, 2004, 1226-1230

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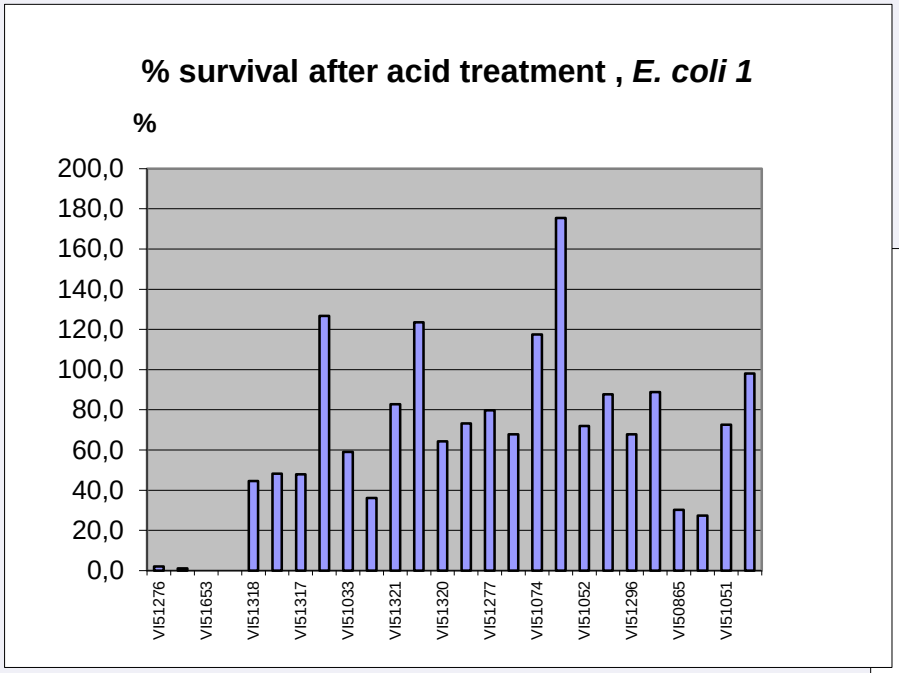
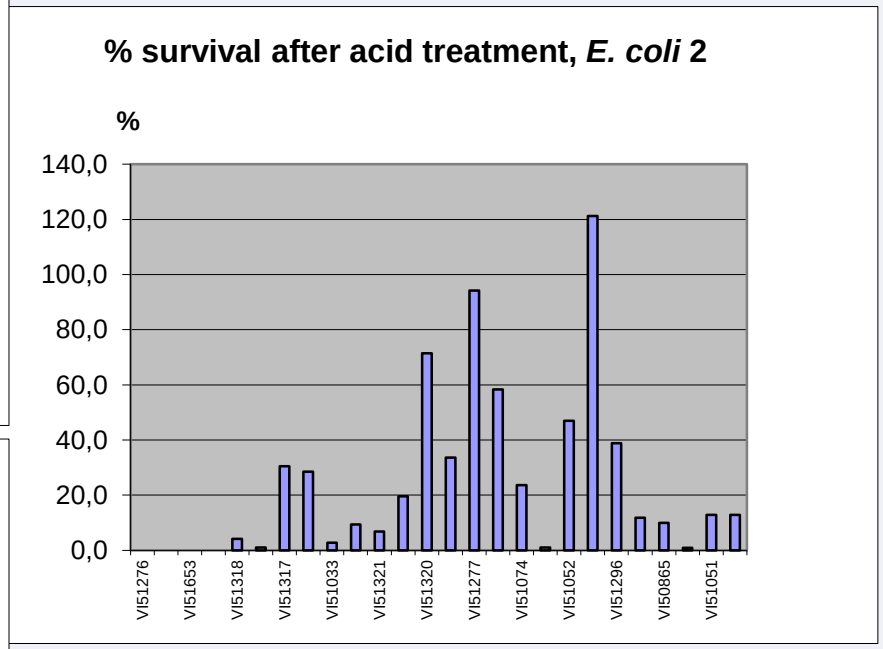
Testing in our laboratory

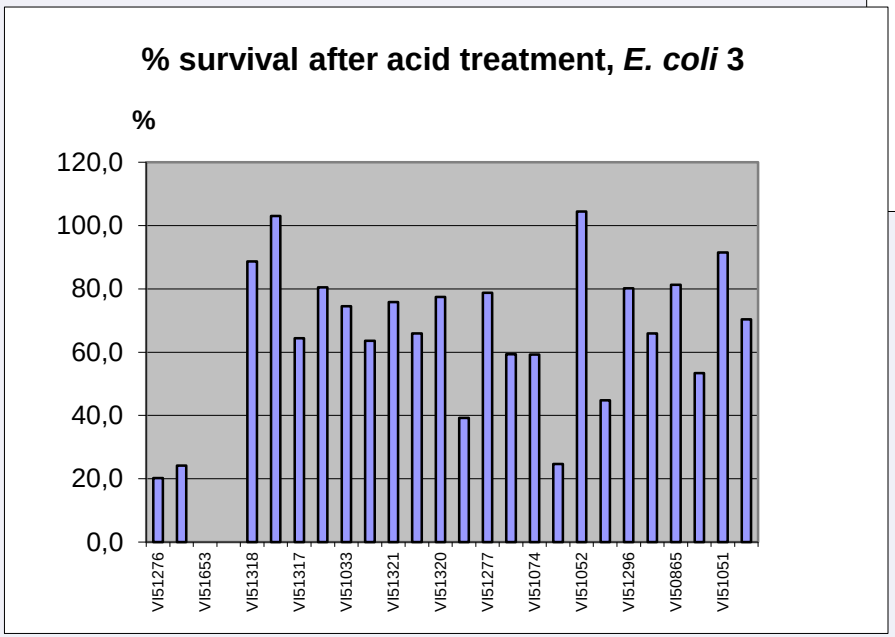
Isolate	Strain ID	Virulence genes	Source
E. coli O8:H9	VI50863	LT1, ST1b	Clinical
E. coli O113	VI51033	stx2+, eae-	Minced meat
E. coli O91	VI51051	stx1+, eae-	EURL ring trial
E. coli O26	VI51052	eae+	EURL ring trial
E. coli O174	VI51074	stx1 and 2+,eae-	Sheep feces
E. coli	VI51276	Control strain	CCUG 17620
E. coli O157:H7	VI51277	stx2:cat	
E. coli O55	VI51296		CCUG 32968
E. coli O111:H8	VI51317	stx-, eae+	From NIPH
E. coli O111:H-	VI51318	stx-, eae+	From NIPH
E. coli O145:H8	VI51320	stx-, eae+	From NIPH
E. coli O145:H-	VI51321	stx-, eae+	From NIPH
E. coli O104	VI51653		From NIPH

Testing on pure cultures

- Grown pure cultures in TSBYE ON (stationary phase)
- Diluted to approx 100 and 1000 cfu/ml (used different diluents)
- 1 ml of dilution to 9 ml TSB (pH2), 30 min@ room temp.
- 1 ml of acidified TSB to 9 ml fresh TSBYE, 37°C over night
- Plating on Blood agar
- Calculated cfu/ml before and after acidification







Variation between strains
E. coli O104 does not survive
Control strain survives in 2/3 rounds

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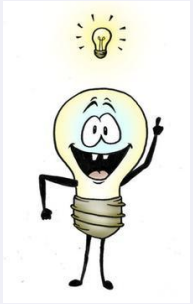
EU RL PT 15 Detection of VTEC in sprouts

- ISO TS 13136 was followed as described
  - Enrichment in BPW, 37°C approx 18 hrs,
  - PCR positive for O111, eae and stx2 on 2/3 samples
  - AIMS for O111 followed by plating on SMAC, Chrom Agar O157 and Chrom Agar STEC
  - Picked and pooled and PCR-ed 50 colonies – all negative!
  - Picked and pooled and PCR-ed another 50 colonies – still all negative☹
  - Agglutinated all 100 colonies with O111 antiserum, 1 positive, but negative when re-PCR-ed (probably *Morganella*?)

We test the acid treatment!



- Enrichment broth from fridge was resuscitated (1 ml enrichment – 9 ml fresh BPW, 37°C for 5 hrs)

Enrichment

Centrifuge 1 ml, 12000 x g, 3 min

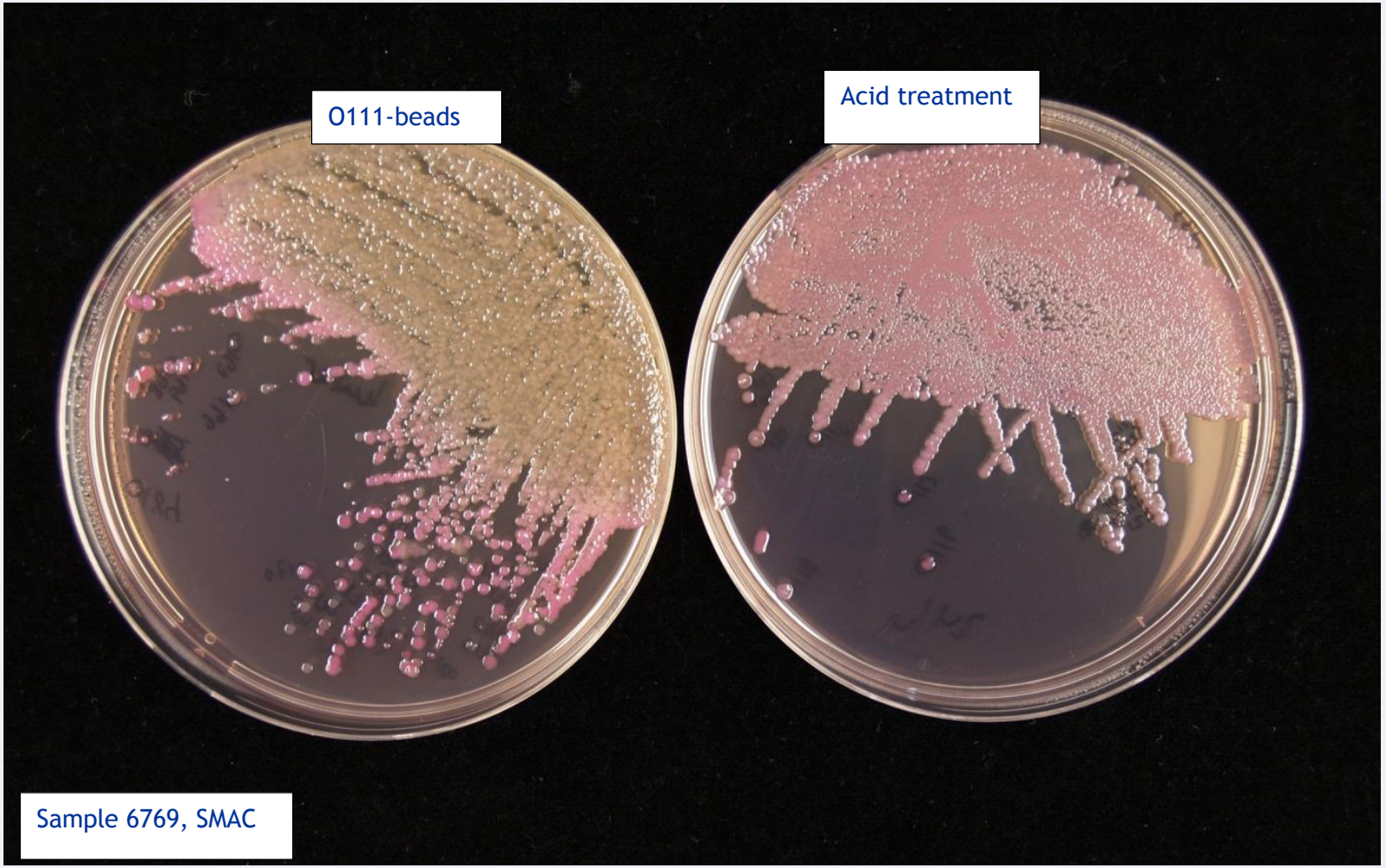
AIMS for O111

Plating on SMAC, Chrom O157 and Chrom STEC

Resuspend in TSB (pH2), incubate 30 min, room temp

Centrifuge 12000 x g, 3 min, resuspend in TSBYE

Results



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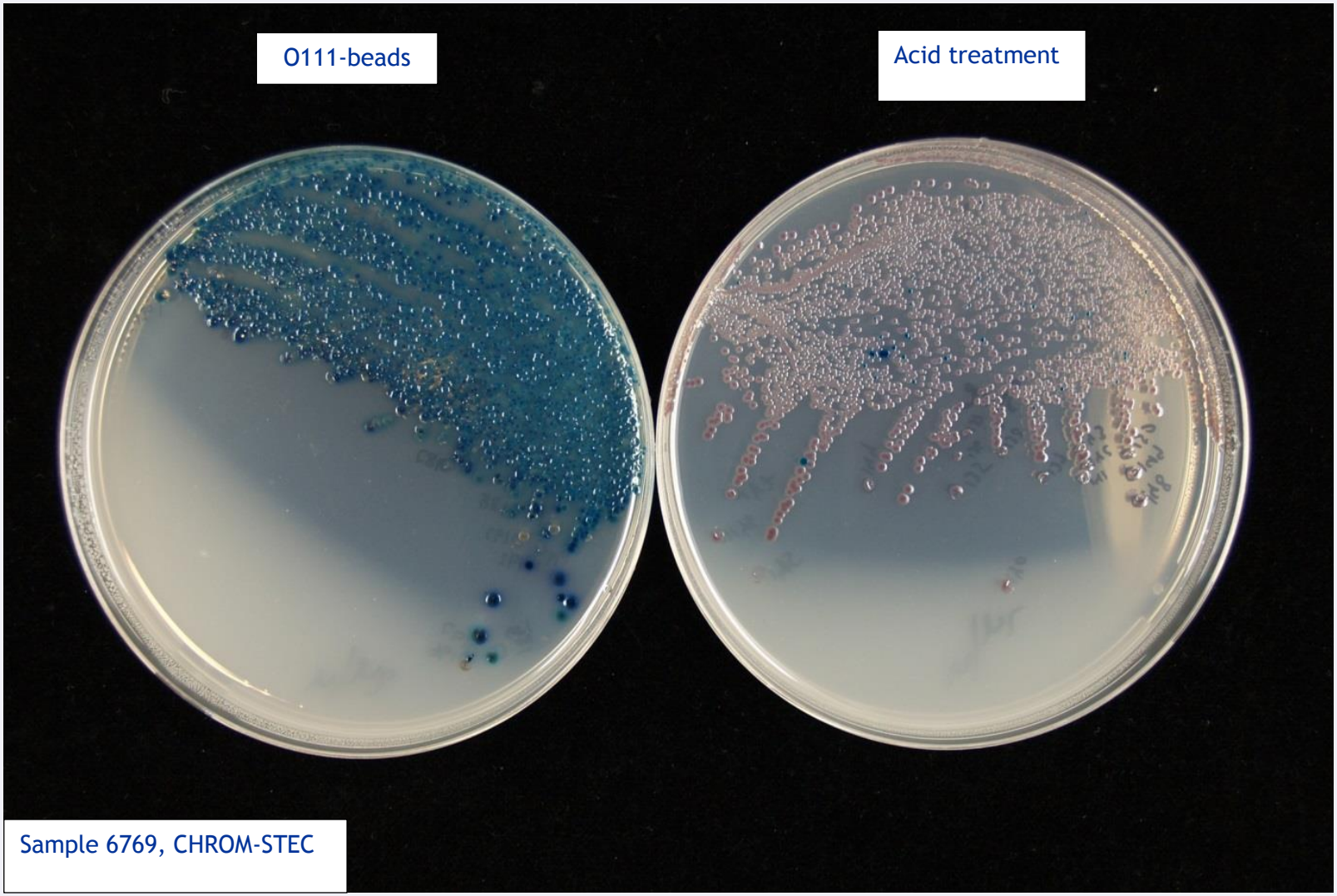
Results cont.



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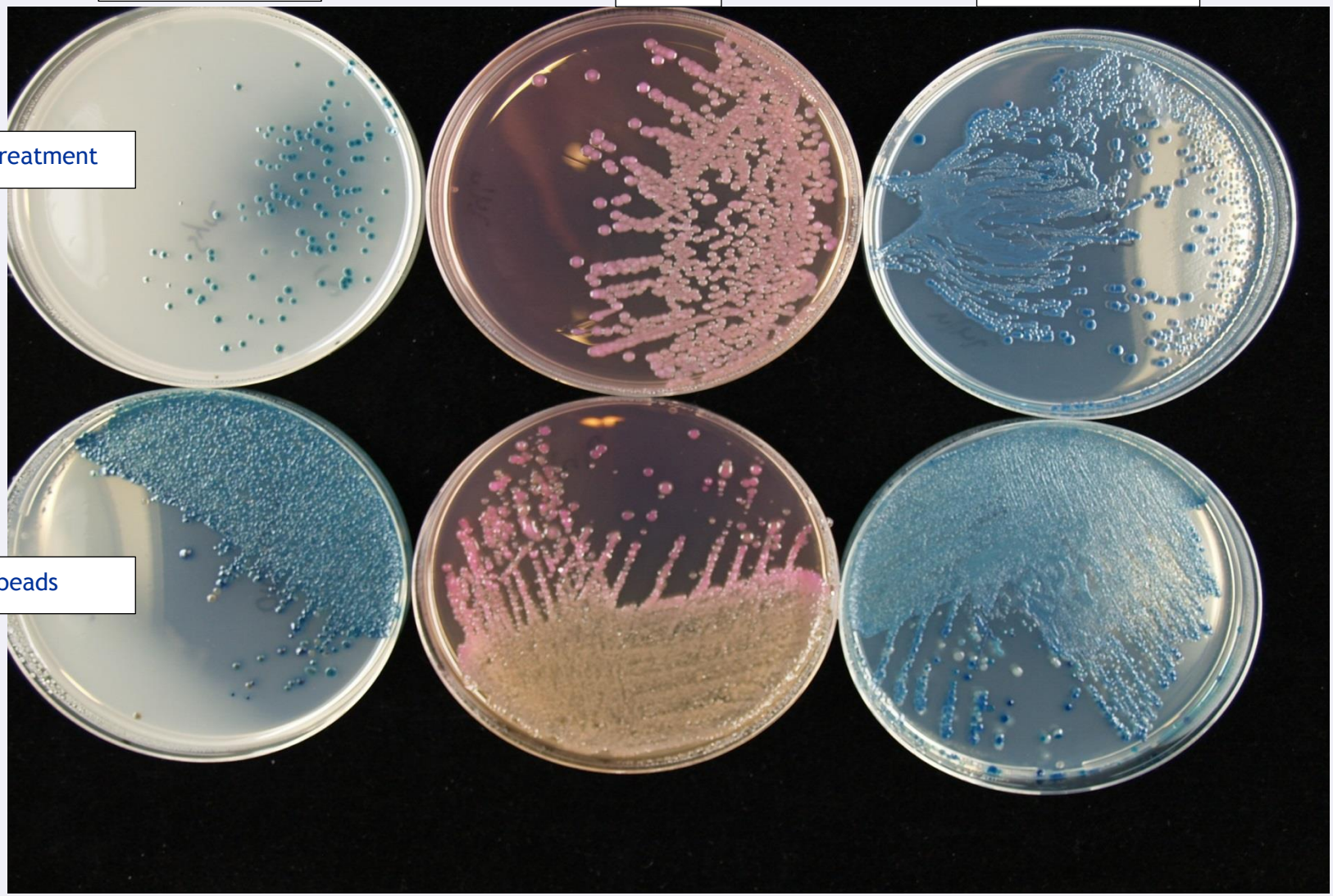
Results – cont.

Sample 5729



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Confirmation from plates

Acid treatment

AIMS O111

	Prøve 1 (6769)				
Opprinnelse		O111	eae	stx1	stx2
Pooler av kolonier plukket fra syrebehandlet oppformert buljong	1_pool 11	21,31	20,6	24	22,67
	1_Pool-12	21,27	20,19	21,96	22,3
	1_Pool-13	20,62	19,61	21,6	21,79
	1_Pool-14	19,37	18,37	No Ct	20,64
	1_Pool-15	20,27	18,73	No Ct	21,11
	1_pool 11_1:10	24,21	23,87	39,77	26,02
	1_Pool-12_1:10	24,06	23,07	23,79	25,73
	1_Pool-13_1:10	24,08	22,55	23,45	24,86
	1_Pool-14_1:10	No Ct	21,27	22,79	23,57
	1_Pool-15_1:10	22,61	21,94	23,34	24,12
	1_kol 145	No Ct	20,46	21,5	22,54
	1_kol 145_1:10	24,29	23,74	25,26	25,78
Pooler av kolonier plukket fra IMS O111 oppkonsentrert fra oppformert buljong	1_Pool-16	No Ct	No Ct	No Ct	No Ct
	1_Pool-17	No Ct	No Ct	No Ct	No Ct
	1_Pool-18	No Ct	No Ct	No Ct	No Ct
	1_pool 19	No Ct	42,04	No Ct	39,42
	1_Pool-20	27,67	25,92	27,89	26,14
	1_Pool-16_1:10	No Ct	No Ct	No Ct	No Ct
	1_Pool-17_1:10	No Ct	No Ct	No Ct	No Ct
	1_Pool-18_1:10	No Ct	No Ct	No Ct	No Ct
	1_pool 19_1:10	No Ct	41,31	No Ct	44,16
	1_Pool-20_1:10	31,33	29,72	43,96	29,84

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Confirmation from plates cont.

Acid treatment

AIMS O111

	Prøve 1 (6769)				
Opprinnelse	Well Name	O111	eae	stx1	stx2
Kolonier plukket fra syrebehandlet oppformert buljong	1_kol101_O111	22,41	21,6	No Ct	23,72
	1_kol103_O111	25,43	23,79	No Ct	23,98
	1_kol102_O111	25,18	24,28	No Ct	24,84
	1_kol104_O111	24,07	23,19	No Ct	23,81
	1_kol105_O111	24,54	23,16	No Ct	24,06
	1_kol106_O111	No Ct	No Ct	No Ct	No Ct
	1_kol107_O111	23	21,41	No Ct	23,08
	1_kol108_O111	25,8	23,95	No Ct	24,78
	1_kol109_O111	21,13	20,82	22,84	22,89
	1_kol110_O111	22,32	21,99	24,52	23,34
Kolonier plukket fra IMS O111 oppkonsentrert fra oppformert buljong	1_kol191_O111	No Ct	No Ct	No Ct	No Ct
	1_kol192_O111	No Ct	No Ct	No Ct	No Ct
	1_kol193_O111	No Ct	No Ct	No Ct	No Ct
	1_kol194_O111	No Ct	No Ct	No Ct	No Ct
	1_kol195_O111	No Ct	No Ct	No Ct	No Ct
	1_kol196_O111	No Ct	No Ct	No Ct	No Ct
	1_kol197_O111	No Ct	No Ct	No Ct	No Ct
	1_kol198_O111	No Ct	No Ct	No Ct	No Ct
	1_kol199_O111	No Ct	No Ct	No Ct	No Ct
	1_kol200_O111	42,83	No Ct	No Ct	35,92

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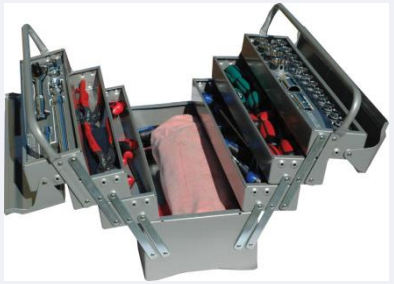
Conclusion – summary



- No miracle treatment, but may be an option for some sample types?
- Cheap, easy and not too time and labour consuming (depending on which approach)
- Strain variations and matrix effects
- Option that should be looked into
- Part of the tool box

Aknowledgement

- Tone Mathisen Fagereng at section for food bacteriology

- To you – for listening

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