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Growth of *Listeria monocytogenes* in Presence of *Listeria innocua* during Traditional Detection Method

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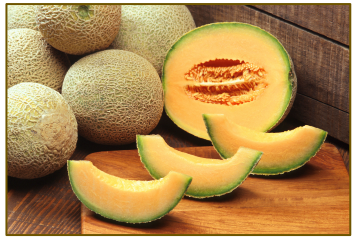
Introduction – *Listeria monocytogenes*

- Public health risk
- 1476 cases in EU in 2011
- 1600 cases per year in USA
- RTE foods: dairy, fishery products, fermented sausage
- Increasing consumer demand for less treated products
- Occurrence of other *Listeria* species in food
- Several detection methods







Growth of *Listeria monocytogenes* in Presence of *Listeria innocua* during Traditional Detection Method

Introduction – Detection method

Numerous scientific literatures discuss problems

- Fraser enrichment broth
  - Acriflavine inhibits *L. monocytogenes*
  - *L. innocua* had inhibitory effect against *L. monocytogenes*
- Agar *Listeria* Ottaviani and Agosti (ALOA) has to be used – ISO 11290-1:1996/A1
  - Non-*L. monocytogenes* colonies can be overlapped by *L. monocytogenes* halos
  - *L. monocytogenes* strains were able to produce halo only after 96 hours

Purpose

Different mixtures of *L. monocytogenes* strains and *L. innocua* strain:

- Growth ability in enrichment steps
- Inhibitory effect of *L. innocua*

Methods

Strains:

- *L. monocytogenes* CCM 4699 (C1)
- *L. monocytogenes* L4 isolated from cheese product
- *L. monocytogenes* T3 isolated from unknown food product
- *L. innocua* CCM 4030 (C6)

Enrichment study:

- Different mixtures were prepared
- Half Fraser broth (hFB) and Fraser broth (FB) used for enrichment steps

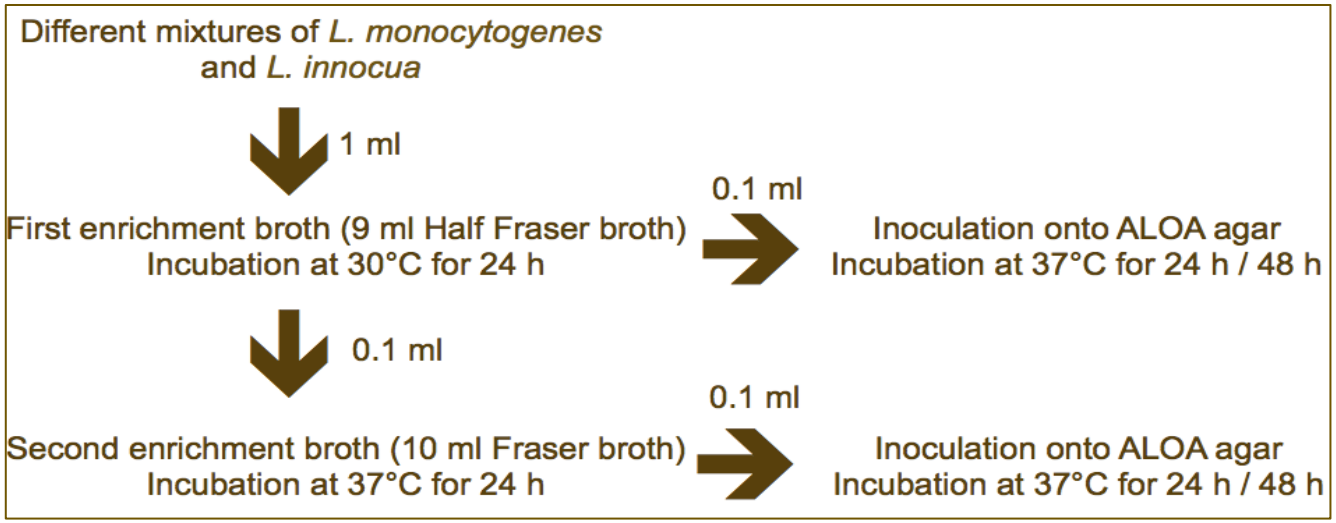
Combination of *L. monocytogenes* L4, T3, C1 and *L. innocua* C6

L. monocytogenes	*L. innocua*
1	1
1	10
1	100

Methods - Competitive growth

- Monocultures and co-cultures of *L. monocytogenes* T3 and *L. innocua* C6
- Growth parameters determined in hFB and FB by DMFit software
- Generation time: GT=0.693/μ

Flowchart of the enrichment steps

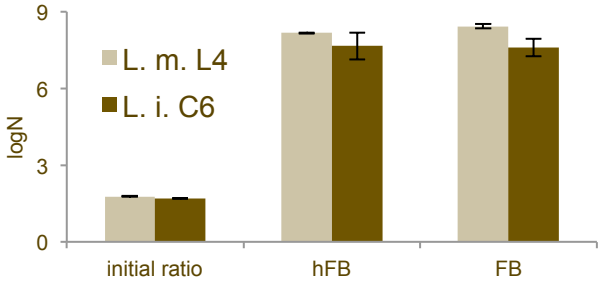


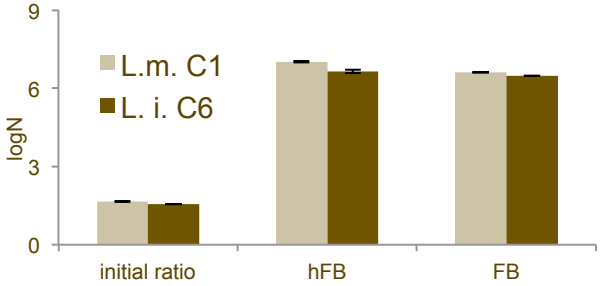
All microbiological measurements were carried out in triplicates.

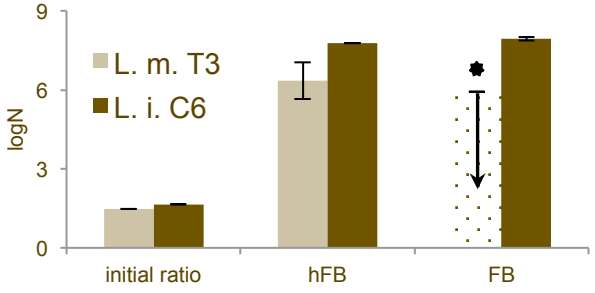
Results – Enrichment study

When the initial ratio was equal:

- *L. monocytogenes* L4 and C1 ratio did not change
- In case of *L. monocytogenes* T3 inhibition was observed

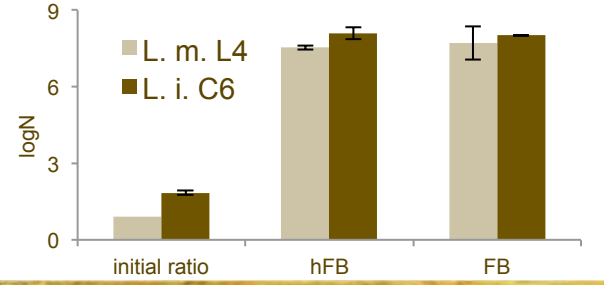


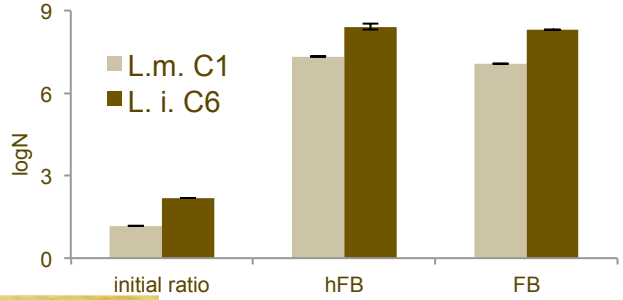


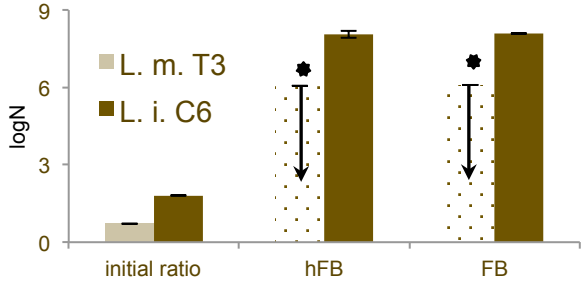


When the initial ratio was 1:10:

- *L. monocytogenes* C1 decreased in hFB and FB
- *L. monocytogenes* L4 no changes
- *L. monocytogenes* T3 could not be detected







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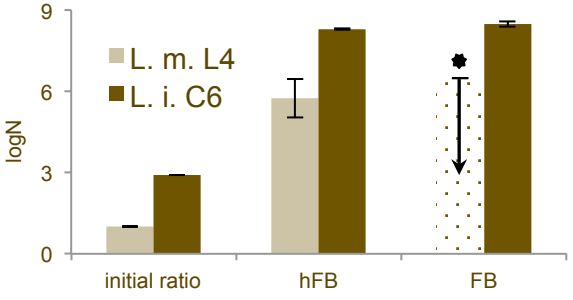
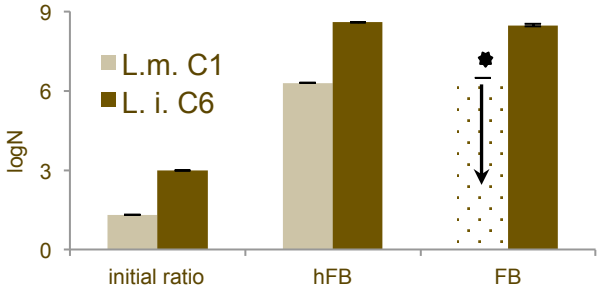
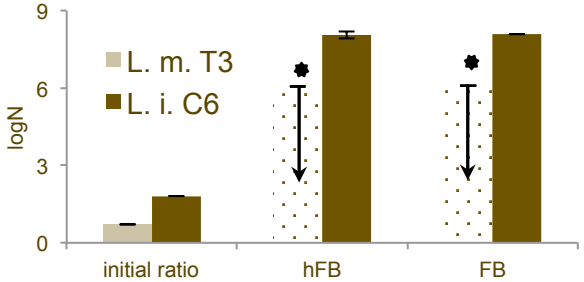
Results – Enrichment study

When the initial ratio 1:100:

- *L. monocytogenes* L4, C1 and T3 could not be detected after the enrichment steps



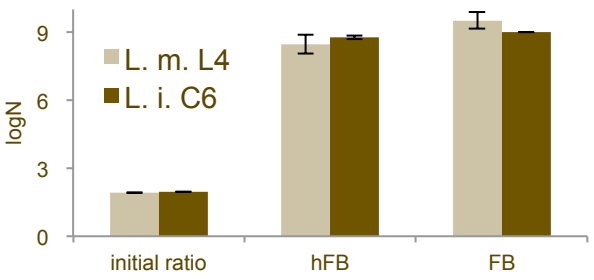


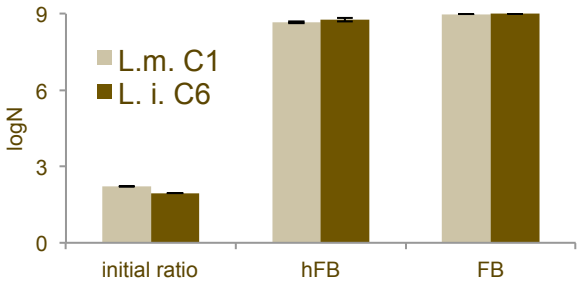


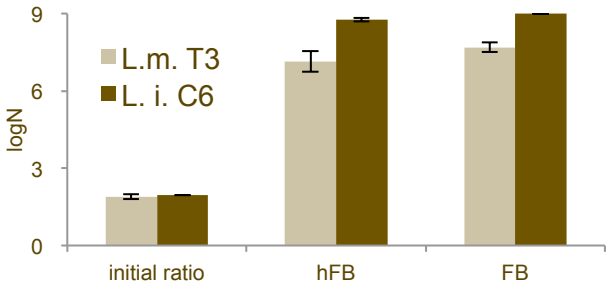
- Better growth ability or adaptation of L. innocua
- Different concentration of acriflavine in hFB and FB
- *L. monocytogenes* is more sensitive for higher acriflavine concentration in presence of *L. innocua*

Results – Enrichment study

- Growth characteristics of the *L. monocytogenes* strains depended on:
  - the initial ratio
  - the strain
- Individual growth:
  - *L. monocytogenes* L4, C1 and *L. innocua* C6 growth ability were same
  - *L. monocytogenes* T3 showed weaker growth







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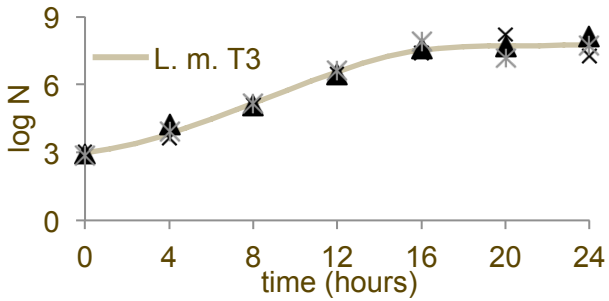
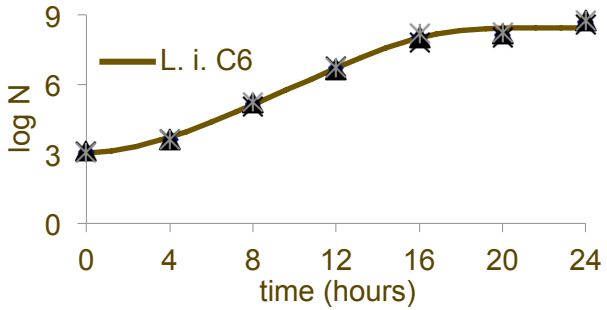
Results – Competitive growth

Mono-cultured *Listeria* strains in hFB

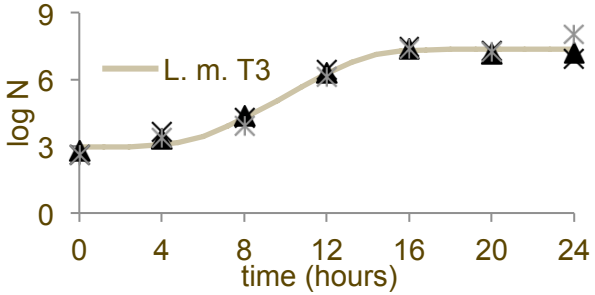
- *Listeria* strains in hFB showed similar tendency
- Colony count of *L. monocytogenes* T3 slightly decreased

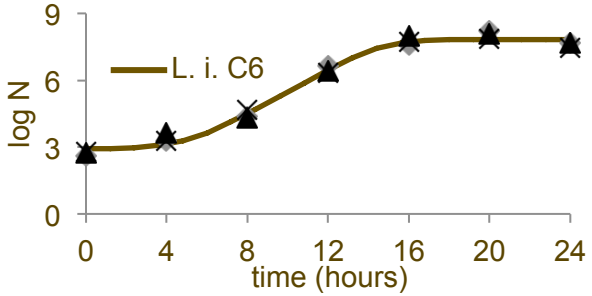




Co-cultured *Listeria* strains in hFB

- Lag phase was prolonged





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Results – Competitive growth

- Lag phase was prolonged in case of *L. monocytogenes* T3
- The generation time was shorter in case of co-cultured *L. monocytogenes* T3

Growth parameters of *L. monocytogenes* T3 and *L. innocua* C6 in hFB

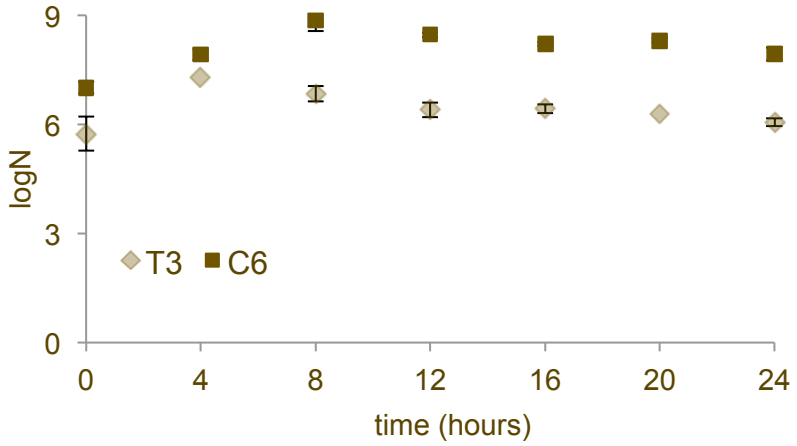
		y_o (logN)	y_{end} (logN)	lag time (h)	growth rate (log unit h^{-1})	r^2	generation time (min)
Mono-cultured	L. m. T3	2.97	7.73	1.7 ± 0.5	0.351 ±0.02	0.998	118
	L. i. C6	3.06	8.44	3.0 ±1.0	0.408 ±0.04	0.991	112
Co-cultured	L. m. T3	2.94	7.37	5.1 ±1.4	0.481 ±0.10	0.978	86
	L. i. C6	2.83	7.86	4.2 ±1.2	0.464 ±0.07	0.986	90

- Competitive growth between *L. monocytogenes* and *L. innocua*
- Quorum sensing interaction?

Results – Competitive growth

Mono-cultured:

- *L. innocua* C6 moderately increased within 8 hours, after the viable cell number slightly decreased
- *L. monocytogenes* T3 cell numbers slightly increased in the first four hours, after decreasing
- End of enrichment steps in FB two-log cycle-difference (lgN_{T3}: 6.0, lgN_{C6}: 7.9)



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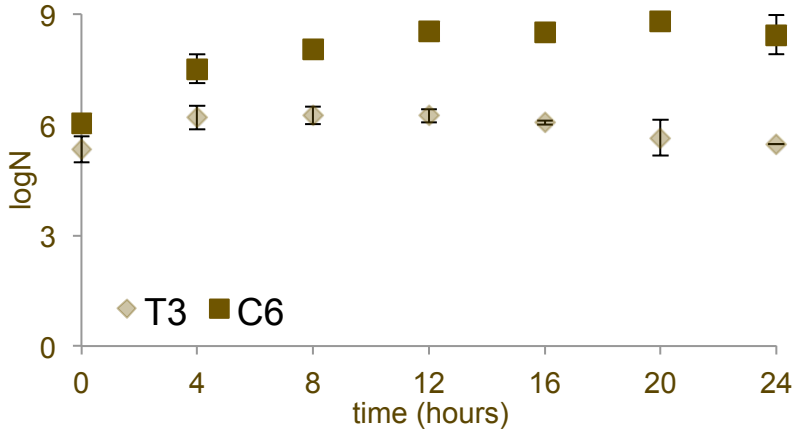
Results – Competitive growth

Co-cultured:

- *L. innocua* C6 cell number increased more than in mono-culture
- *L. monocytogenes* T3 decreasing cell numbers in presence of *L. innocua*
- End of enrichment steps in FB three-log cycle-difference (lgN_{T3}: 5.5, lgN_{C6}: 8.4)



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Results – Competitive growth

- ISO 11290 standard: streaking out from FB must be done after 24±3 hours
  - Viable cell numbers decrease during this interval → lead false negative results
  - No significant increase in *L. monocytogenes* T3 cell number
  - *L. innocua* C6 was able to propagate
- Inhibition of *L. monocytogenes* T3 in co-culture can not simply be explained

Conclusions

- Response of the examined *Listeria monocytogenes* strains:
  - Different during the enrichment protocol
  - Depended on strains
  - Initial ratio
- *L. monocytogenes* T3 was slightly inhibited in hFB by *L. innocua* C6
- In hFB the lag phase was prolonged:
  - With 3.4 hours in case of co-cultured *L. monocytogenes* T3
  - *L. innocua* C6 lag phase was prolonged by 1.2 hours
- *L. monocytogenes* T3 earlier stationary phase could be observed in hFB
- ISO 11290-1:1996 standard is not always reliable

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Thank you for your attention!

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