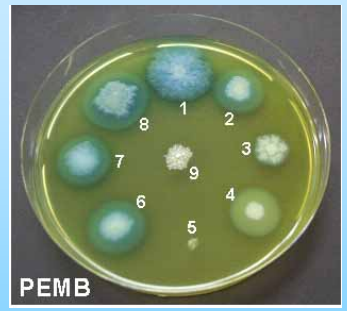
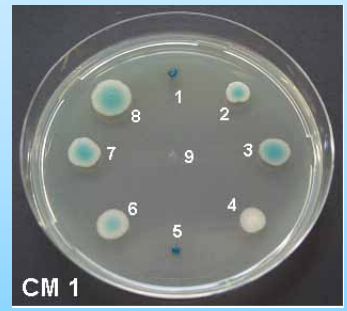
Detection of Food Pathogens using the Smart Cycler II

Martina Fricker, Ingrid Huber, Siegfried Scherer, Monika Ehling-Schulz

Detection of Food Pathogens

- Standard techniques are laborious and time-comsuming
- Not always unequivocal results
- Real-time PCR as emerging technique, also suggested from §35 LMBG
- Saves time
- Less cross-contamination





Outline

Part 1:

Comparison of three Real-time Cyclers with a commercial available kit for detection of *Salmonella* spp.

Part 2:

Recently developed assay for the detection of emetic *Bacillus cereus* strains optimized on the Smart Cycler II

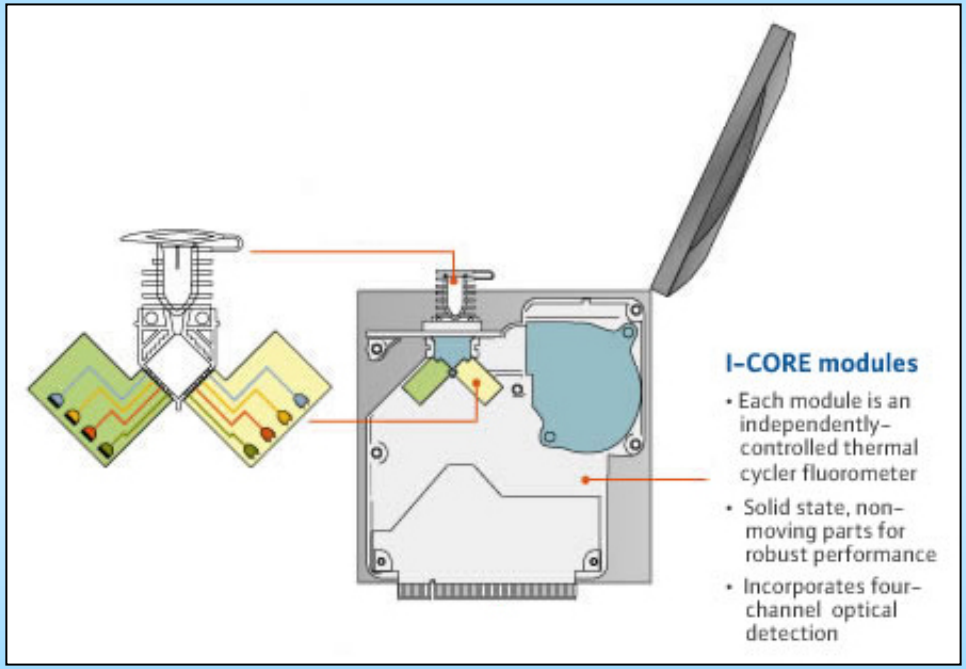
Smart Cycler II – features



- 16 independently programmable reaction sites
- Programmable ramp rate
- Maximum heating rate: 10°C / sec
- Upgradeable to 96 samples by connecting 6 blocks

Smart Cycler II – working principle





For further questions please contact Cepheid in the foyer

Comparison of the tested Real-time Cycler

	Cepheid Smart Cycler II	**Bio-Rad iCycler iQ**	**Stratagene Mx3000P**
Source	LED	Tungsten Halogen	Tungsten Halogen
Detector	Silicon photodetectors	CCD camera	PMT scanner
Heating rate	Programmable, up to 10°C / sec	Programmable, up to 3.3 °C / sec	2.5°C / sec
Samples	16, independent; upgradable to 96	96	96

iQ-Check™ *Salmonella* Kit*

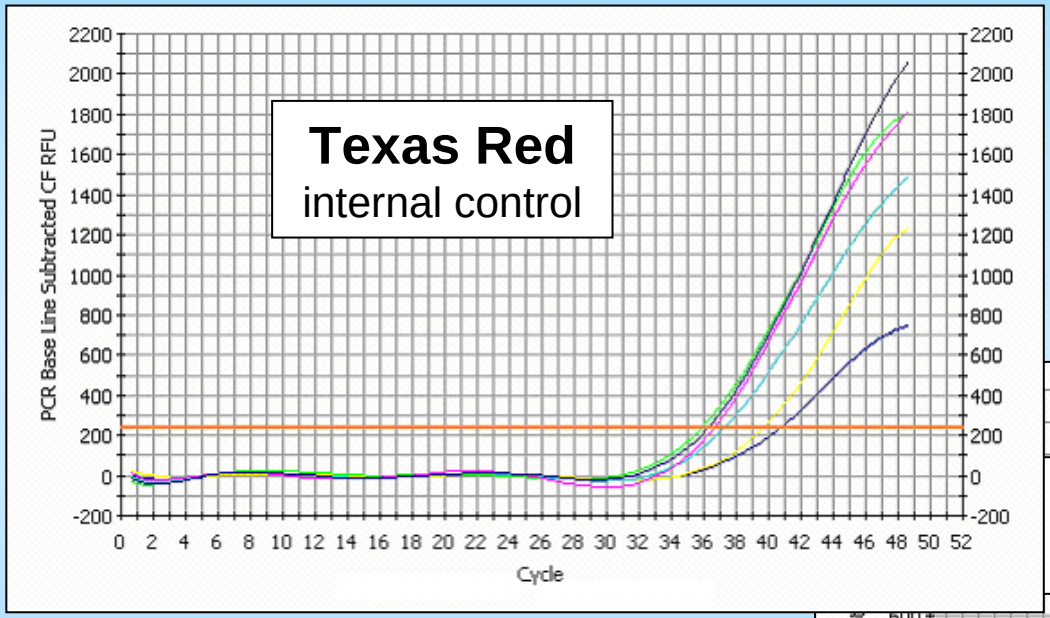
- Real-time PCR detection of *Salmonella* spp. in food
- Detection after enrichment in buffered peptone water
- Molecular Beacons:
  - FAM: probe for *Salmonella* spp. specific DNA sequence
  - Texas Red: internal control

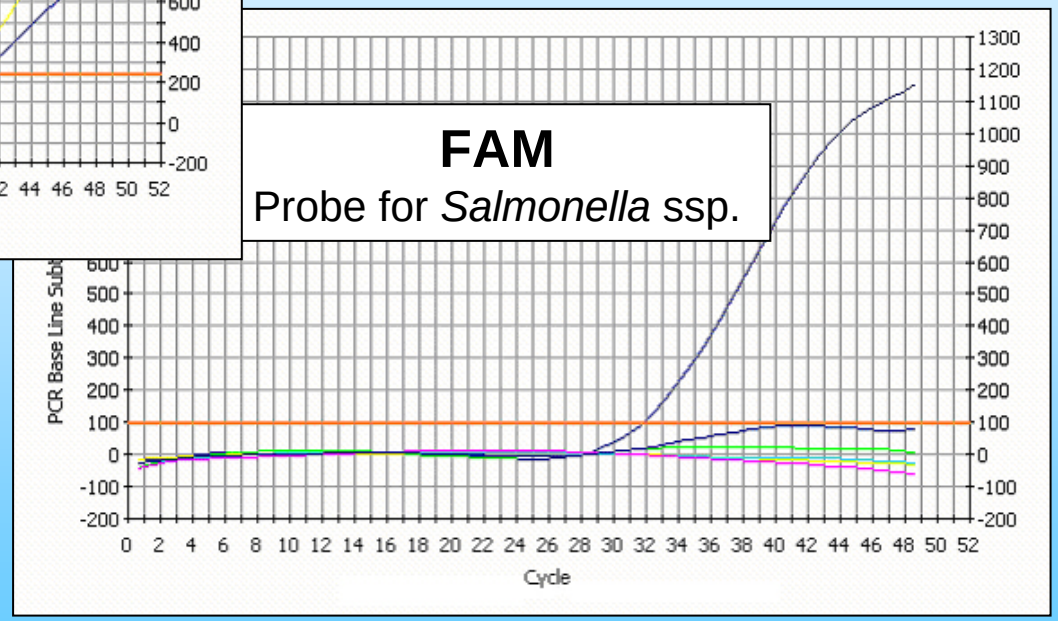
*Bio-Rad

Experimental conditions

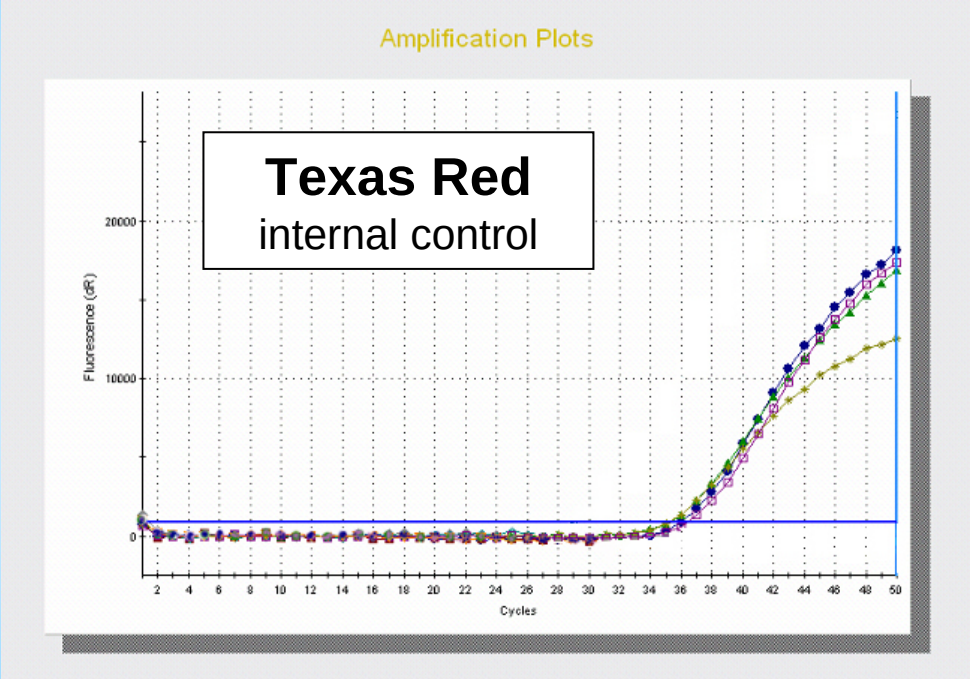
- Comparison of three Real-time Cyclers
  - Bio-Rad iCycler iQ
  - Stratagene Mx3000P
  - Cepheid Smart Cycler II
- Protocol according to kit manufacturer
- Additionally: fast protocol tested in the Smart Cycler II

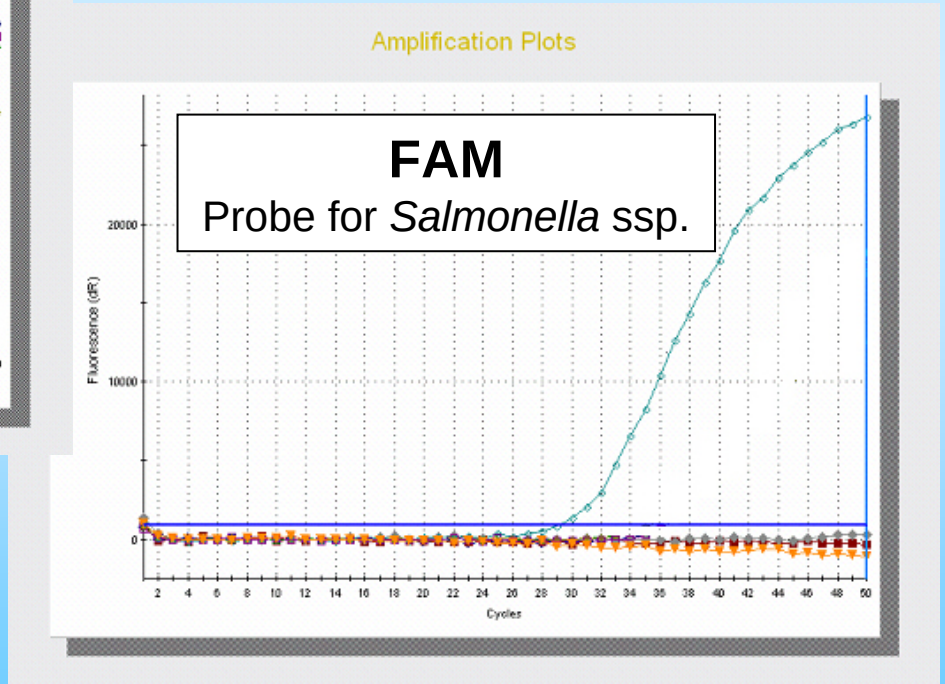
Results with the Bio-Rad iCycler iQ





Results with the Stratagene Mx3000P

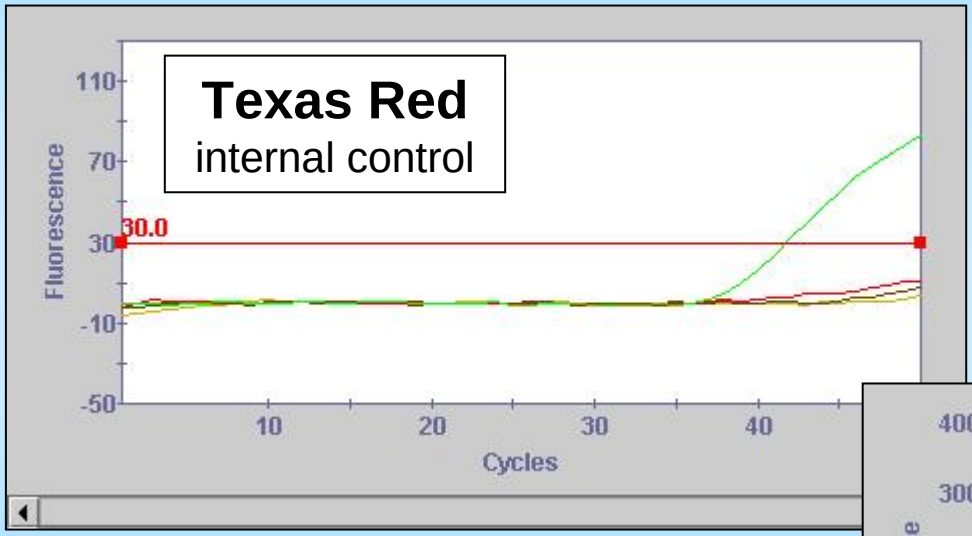


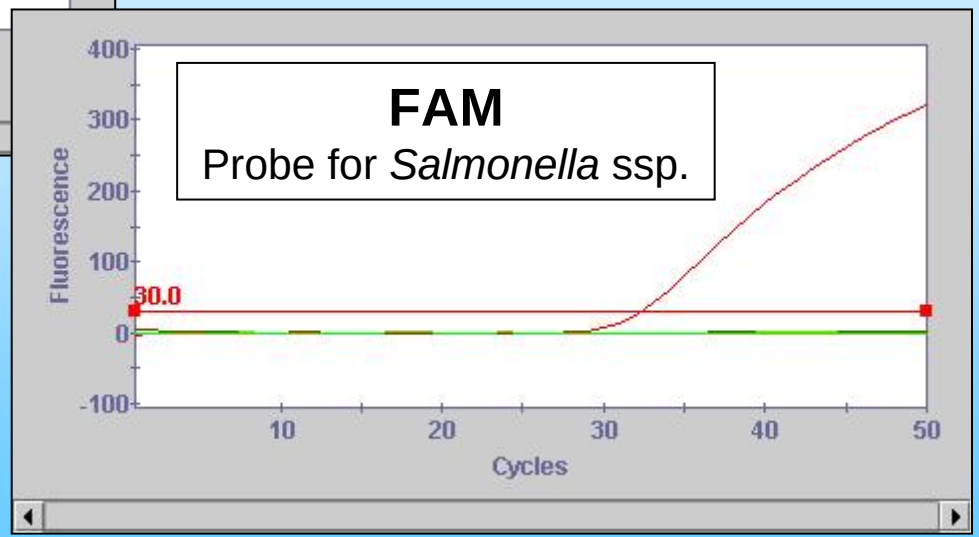


Results with the Smart Cycler II



"**Fast**" protocol: maximal ramp rate (10°C / sec)

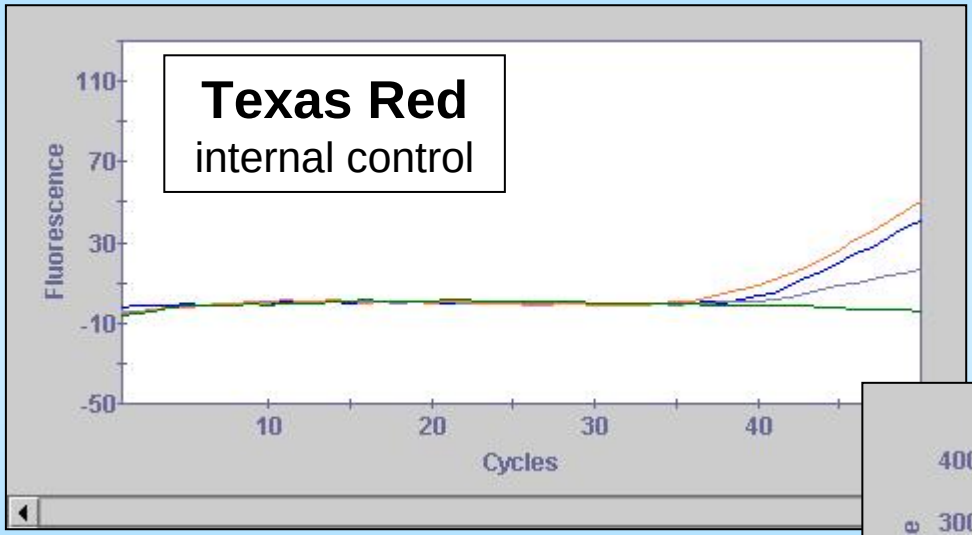


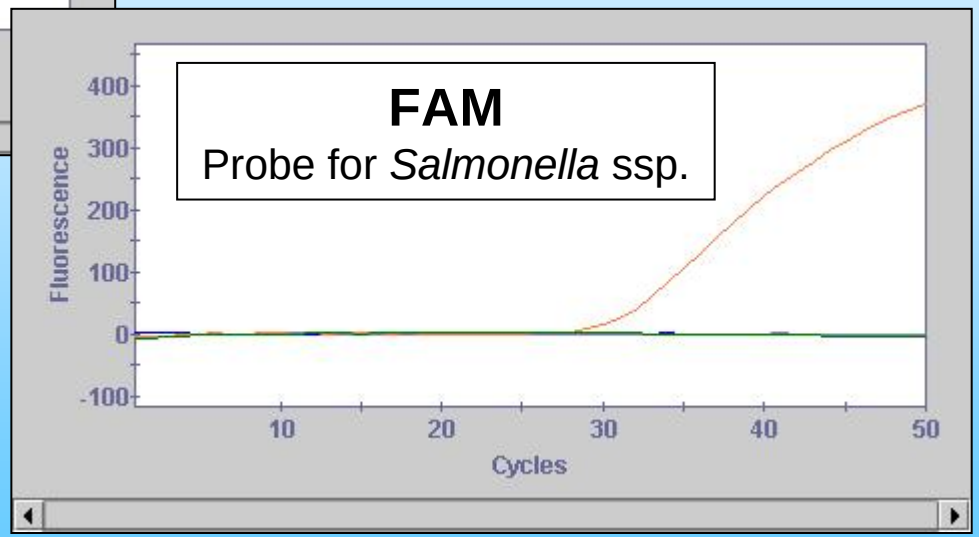


Results with the Smart Cycler II



"**Slow**" protocol: ramp rate of the Bio-Rad iCycler





Conclusions – Part 1

- Bio-Rad iCycler iQ and Stratagene Mx3000P show similar results
- Cepheid Smart Cycler II: Problems with fast ramp rate
  - Chemistry not compatible?
- **But:**
  Smart Cycler II can mimic the conditions of other Real-time cyclers

⇒ Kits / Chemistries adapted to slow ramp rates can be used in the Smart Cycler II

Overview

Part 1:

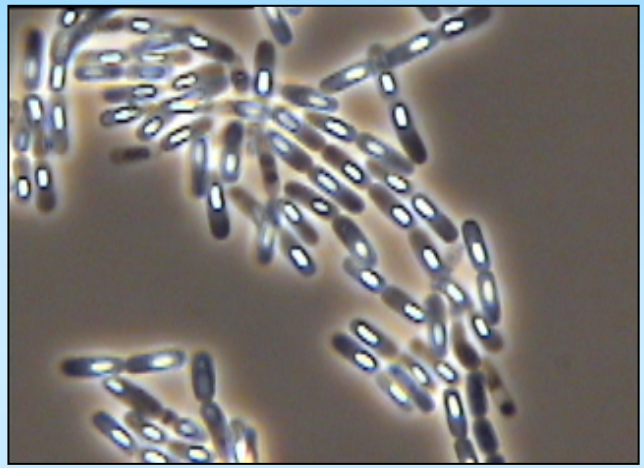
Comparison of three Real-time Cyclers with a commercial available kit for detection of *Salmonella* spp.

Part 2:

Recently developed assay for the detection of emetic *Bacillus cereus* strains optimized on the Smart Cycler II

Bacillus cereus

- Gram positive
- Motile
- Rod shaped
- Facultative anaerobic
- Spore forming
- Ubiquitous
- Growth range from 10°C to 50°C



- *Bacillus cereus* group

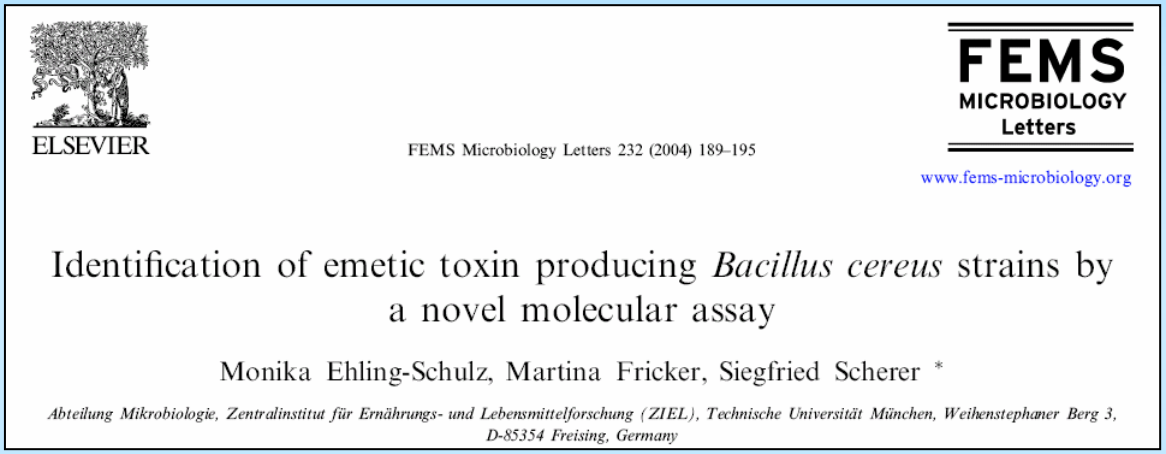
  (*B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*)

Toxins and symptoms

- Enterotoxins: HBL, NHE, cytK
  - Symptoms: abdominal pain, diarrhea
  - Implicated food: meat products, soups, vegetables, puddings, milk and milkproducts
- Emetic Toxin: Cereulide
  - Symptoms: nausea and vomiting
  - Implicated food: fried and cooked rice, pasta, pastry

PCR detection of emetic *B. cereus* strains

- We developed specific PCR assays



ELSEVIER

FEMS Microbiology Letters 232 (2004) 189–195

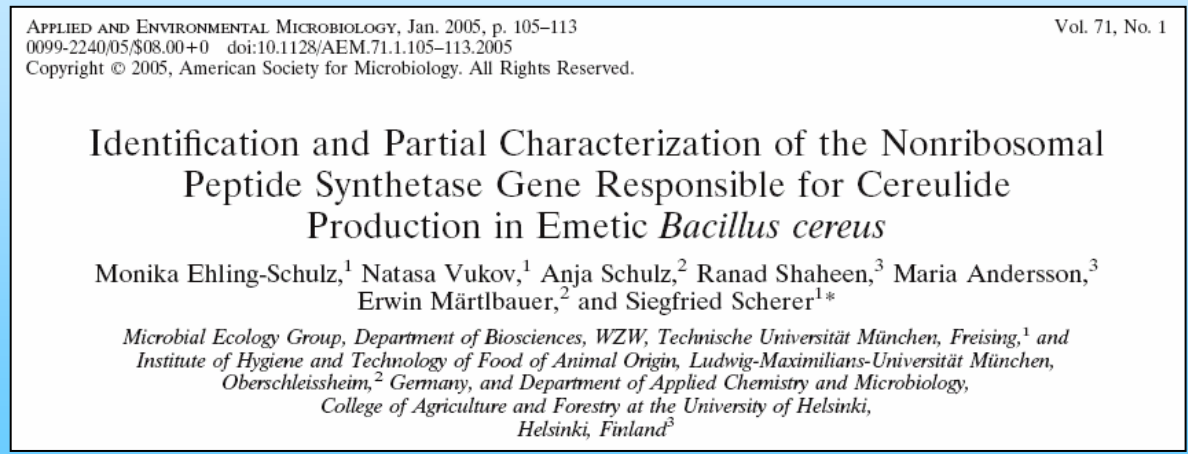
FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Identification of emetic toxin producing *Bacillus cereus* strains by a novel molecular assay

Monika Ehling-Schulz, Martina Fricker, Siegfried Scherer *

Abteilung Mikrobiologie, Zentralinstitut für Ernährungs- und Lebensmittelforschung (ZIEL), Technische Universität München, Weihenstephaner Berg 3, D-85354 Freising, Germany



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2005, p. 105–113 Vol. 71, No. 1
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.1.105–113.2005
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Identification and Partial Characterization of the Nonribosomal Peptide Synthetase Gene Responsible for Cereulide Production in Emetic *Bacillus cereus*

Monika Ehling-Schulz,[1] Natasa Vukov,[1] Anja Schulz,[2] Ranad Shaheen,[3] Maria Andersson,[3] Erwin Märtlbauer,[2] and Siegfried Scherer[1]*

Microbial Ecology Group, Department of Biosciences, WZW, Technische Universität München, Freising,[1] and Institute of Hygiene and Technology of Food of Animal Origin, Ludwig-Maximilians-Universität München, Oberschleissheim,[2] Germany, and Department of Applied Chemistry and Microbiology, College of Agriculture and Forestry at the University of Helsinki, Helsinki, Finland[3]

PCR detection and enrichment

Homogenisation of cooked rice with BHIG

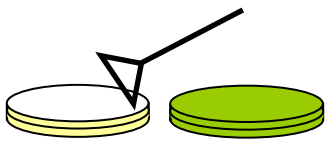
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Inoculation of aliquots with different amounts of F4810/72

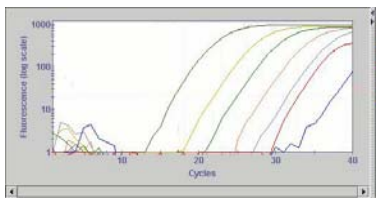
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Enrichment at 37°C

↓

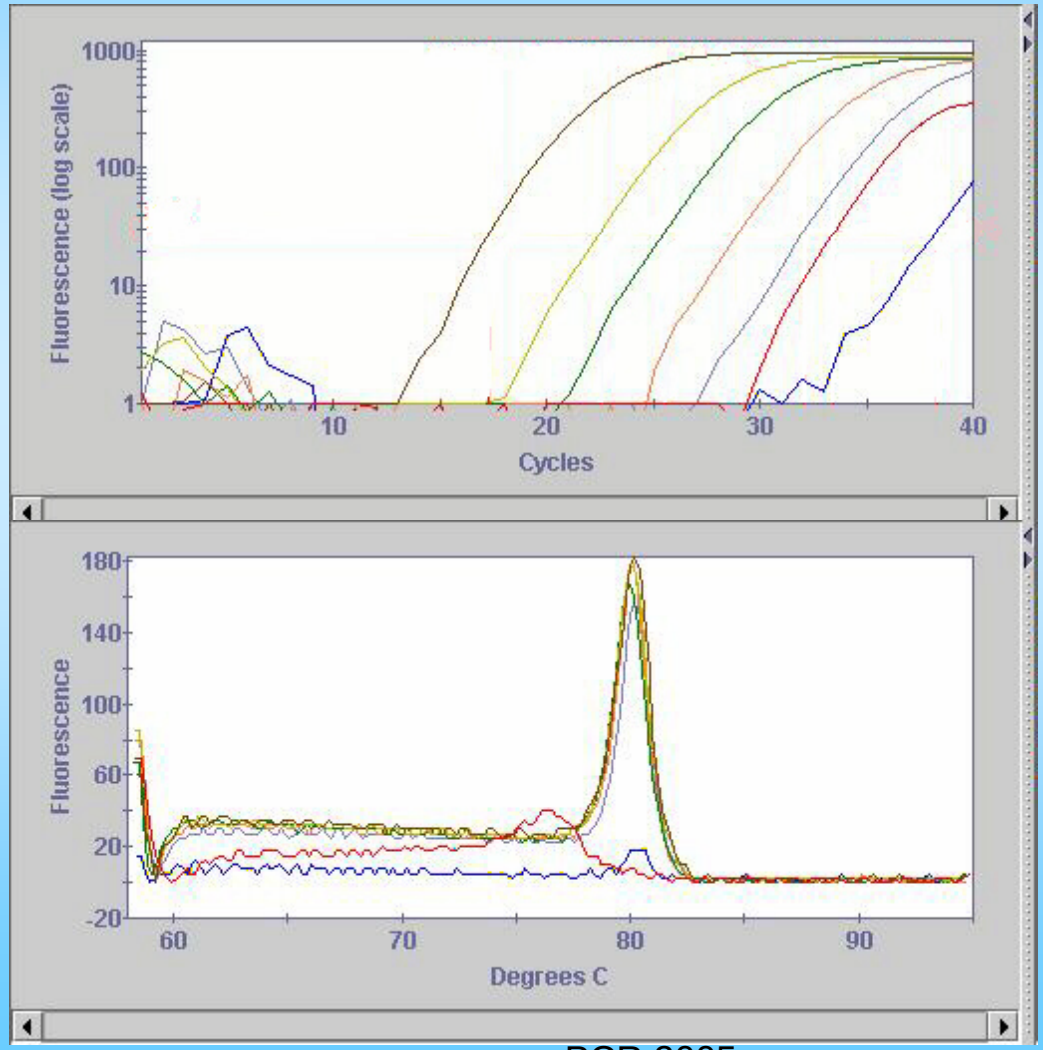


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Plating of serial dilutions on LB / PEMB agar and Real-time PCR

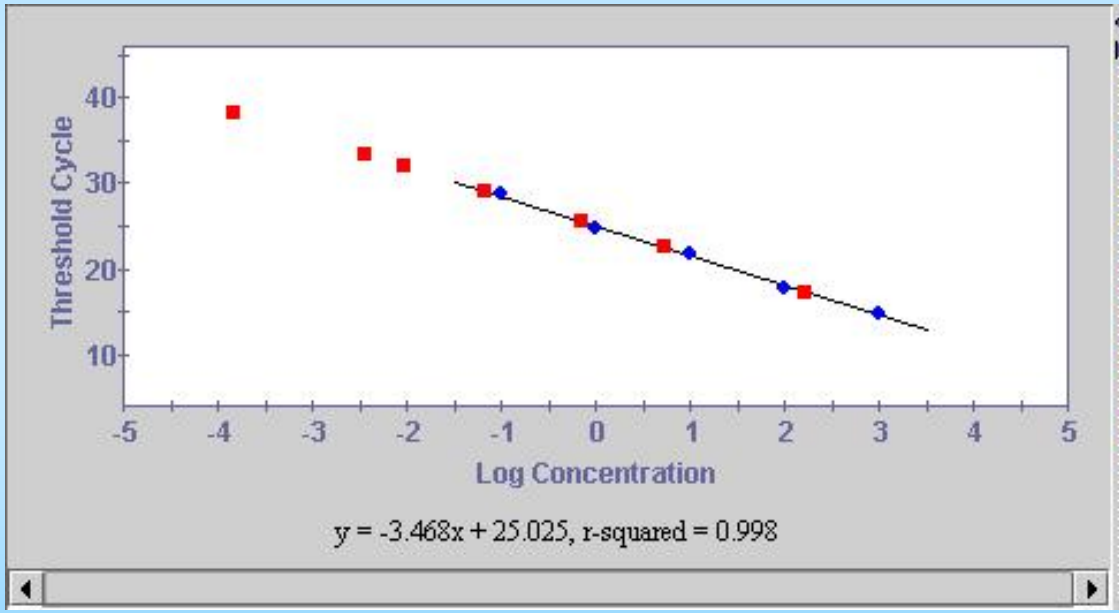
Real-time PCR of rice samples with SybrGreen



- + Control
- 10^3 cfu/g
- 10^2 cfu/g
- 10^1 cfu/g
- 10^0 cfu/g
- NTC
- K4

After 4h enrichment

Real-time PCR experiments with rice

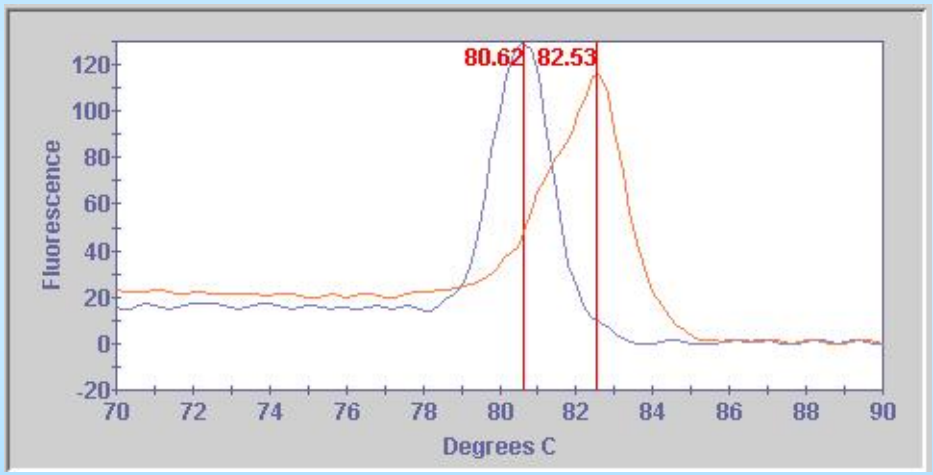


Site ID	Protocol	Sample ID
A9	slow nrps	1000
A10	slow nrps	100
A11	slow nrps	10
A12	slow nrps	1
A13	slow nrps	0.1
A9	slow nrps	K4 (2)
A10	slow nrps	10 0 4h (2)
A11	slow nrps	10 1 4h (2)
A12	slow nrps	10 2 4h (2)
A13	slow nrps	10 2 4h (2)
A14	slow nrps	+ Kontrolle
	slow nrps	NTC

Symptoms of food poisoning with emetic *B. cereus* or *S. aureus*

Species	Infective agent	Incubation time	Symptoms	Duration
***Bacillus cereus* (emetic)**	**toxin**	**1-6 h**	**nausea, vomiting**	**6-24 h**
Staphylococcus aureus	toxin	1-6 h	nausea, vomiting, abdominal pain	8-24 h

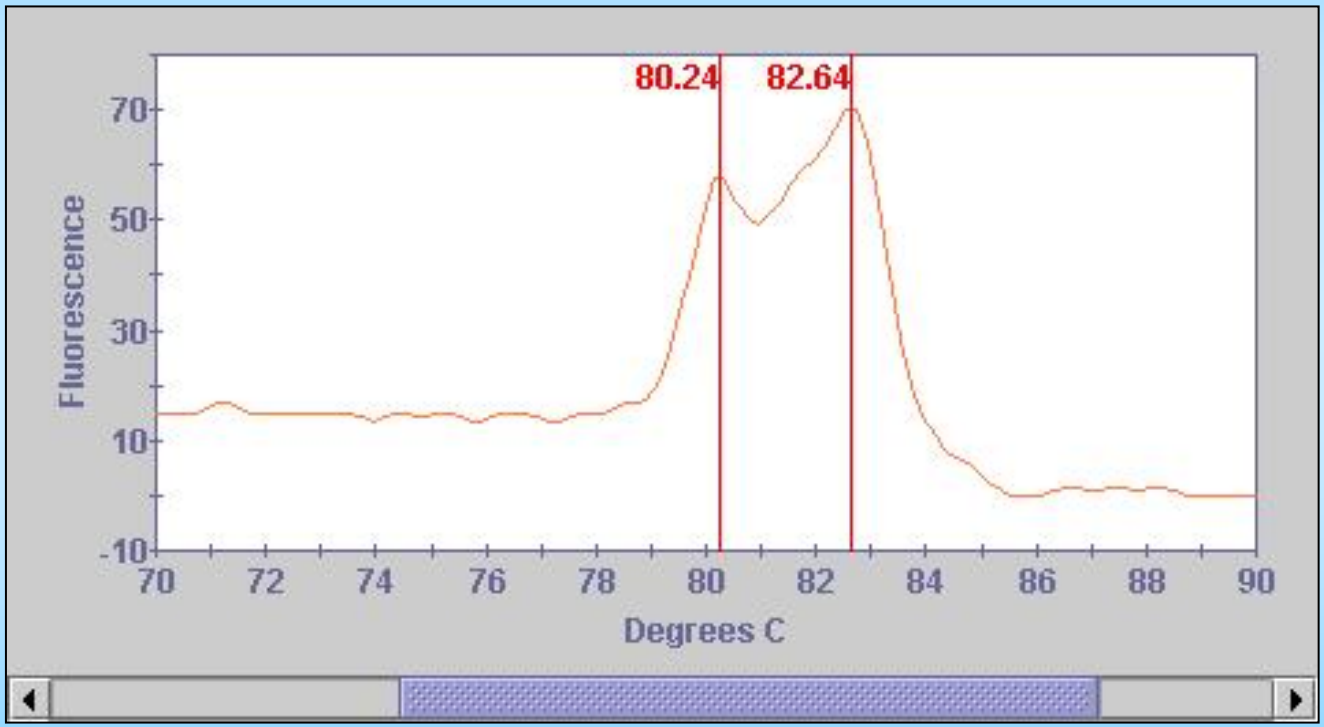
Duplex real time PCR for the differentiation of emetic *B. cereus* and *S. aureus*



T_m of the amplicon for emetic *B. cereus* (80,73 ± 0,25°C)

T_m of the amplicon for *S. aureus* (82,62 ± 0,35°C)

Results for duplex Real-time PCR



emetic *B. cereus* (80,35 ± 0,4°C)

S. aureus (82,33 ± 0,46°C)

Conclusions – Part 2

- Detection limit: 10^3 cfu / g rice for SYBR Green assay
- Taqman assay still has to be optimized (other chemistry…)
- Duplex Real-time PCR is possible for the one step differentiation of emetic *B. cereus* and enterotoxigenic *S. aureus*