Detection of *Staphylococcus aureus* and *Listeria monocytogenes* DNA in artificially contamined spices

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Location: Berlin, BfR

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement n° 312631.

- BIOR – supporting partner in task 3.5 of work package 3 **Development and evaluation of rapid qualitative on-site detection/screening methods for biological contaminants** leading by VUP

- Tasks:

✓ Contamination of spices and herbs samples with known concentration of bacterial pathogen (*Staphylococcus aureus, Listeria monocytogenes*)

✓ Direct bacterial DNA extraction from spices and herbs using CTAB (cetyltrimethylammoniumbromide) protocol

✓ Detection of bacterial DNA presence in samples by PCR

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CTAB DNA extraction

Type of samples:
plant, bacteria, protozoa, fungi, insects, animal

Amount of starting material: µgs – several grams

+ t°C
+ Proteinase K
+ Rnase
+ β-mercapthoethanol
+ Polyvinylpirrolidone (PVP)

CTAB + low salt conditions

Advantages: low costs, adjustable for a broad type of samples
Disadvantages: laborious, time consuming

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Staphylococcus aureus

- Gram (+), facultative anaerobe, non motile, non sporulate;
- Resistant to high salt conditions and desiccation;
- Common inhabitant of the skin and nasopharynx of humans and animals;
- Produces heat stabile enterotoxins;
- *S. aureus* start to produce enterotoxins at concentration of $>10^5$ CFU/g;
- EU Regulation No 2073/2005 lays down food safety criteria for staphylococcal enterotoxins in dairy products:
  - Diary products must be tested for enterotoxins if coagulase-positive staphylococci are detected at levels $>10^5$ CFU/g.

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Detection of *Staphylococcus aureus*

•Each matrix sample (parsley, basil, cinnamon, vanilla) was contamined with different concentration (10^2 to 10^6 CFU per gramm) of *S. aureus;*

•DNA extracted with CTAB protocol from sample amount 0.5g;

•A protocol for **16S rRNA** gene specific for *S. aureus* was used
MASON W.J, BLEVINS J.S, BEENKEN K., WIBOWO N., OJHA N., SMELTZER M.S **Multiplex PCR Protocol for the Diagnosis of Staphylococcal Infection.** JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2001, p. 3332–3338

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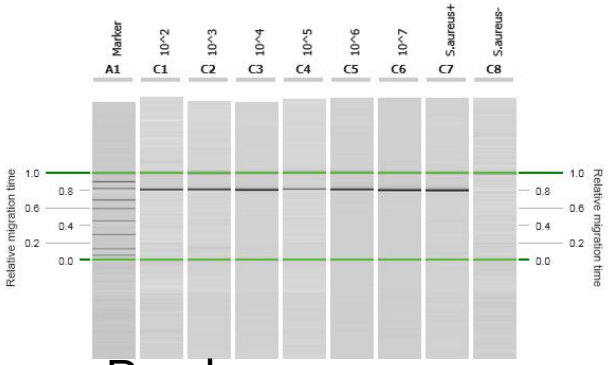
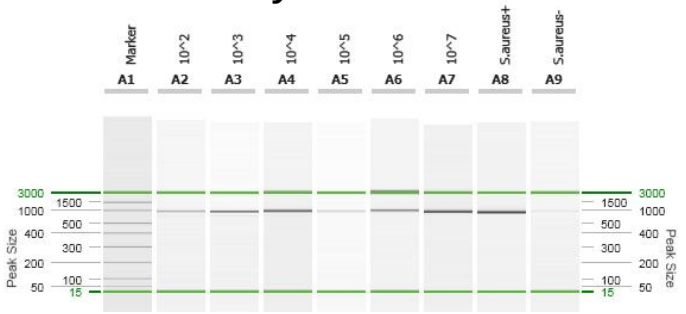
16S rRNA PCR results *S.aureus*

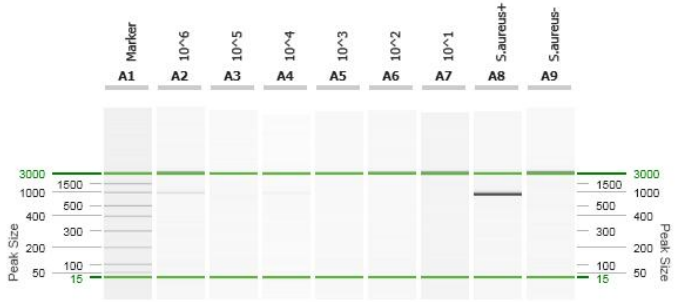
- *S.aureus* DNA was dectetable down to 10^2 CFU/g in three matrices – parsley, basil, vanilla
- In cinnamon *S.aureus* DNA was dectetable down to 10^4 CFU/g



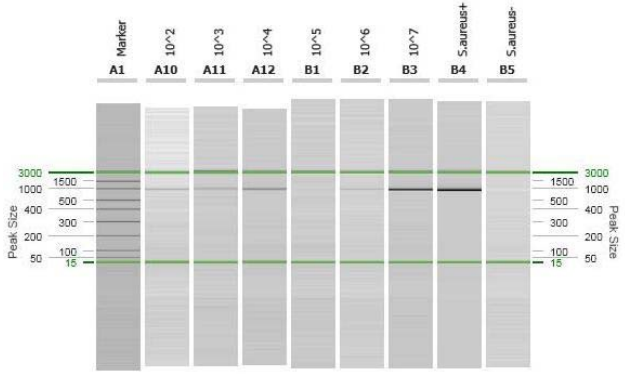
Parsley



Basil



Cinnamon



Vanilla

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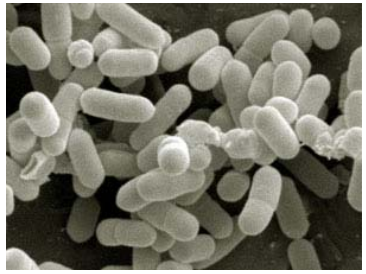
Listeria monocytogenes



- Ubiquitous food-borne pathogen (soil, vegetation, water …), can be found in unprocessed foods of animal origin and ready-to-eat (RTE) foods by post-processing contamination;
- Gram (+), facultative anaerobe, can grow at refrigerator temperatures, resistant for low pH
- EU Regulation No 2073/2005 lays down food safety criteria for *L. monocytogenes* in RTE foods:
  - In RTE food for infants and special medical purposes *L. monocytogenes* must not be present in 25 g,
  - *L. monocytogenes* must not be present in levels above 100 cfu/g during the shelf life of other RTE products.

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Detection of *Listeria monocytogenes*

•Each matrix sample (black pepper, paprika/chili, parsley, oregano, basil, cinnamon, nutmeg, allspice) was contamined with different concentration (10^2 to 10^6 cfu per gramm) of *L. monocytogenes;*

•DNA extracted with CTAB protocol and CTAB + polyvinylpirrolidone from sample amount 0.2g in duplicates;

•A universal bacterial primers for 16S rRNA gene

S. DORN-IN, R.T BASSITTA, K.SCHWAIGER, J. BAUER, C. S. HÖLZEL. **Specific amplification of bacterial DNA by optimized so-called universal bacterial primers in samples rich of plant DNA.** Journal of Microbiological Methods 113 (2015) 50–56

•Listeria monocytogenes **prfA** gene specific primers

A. KÉROUANTON, M.MARAULT, L.PETIT, J.GROUT, T. T. DAO, A. BRISABOIS. **Evaluation of a multiplex PCR assay as an alternative method for Listeria monocytogenes serotyping**, Journal of Microbiological Methods 80 (2010) 134–137

•Conventional and real time PCR detection for **prfA** gene in two replicates for each extraction was performed.

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Detection of Listeria monocytogenes

•A universal bacterial primers for **16S rRNA** gene

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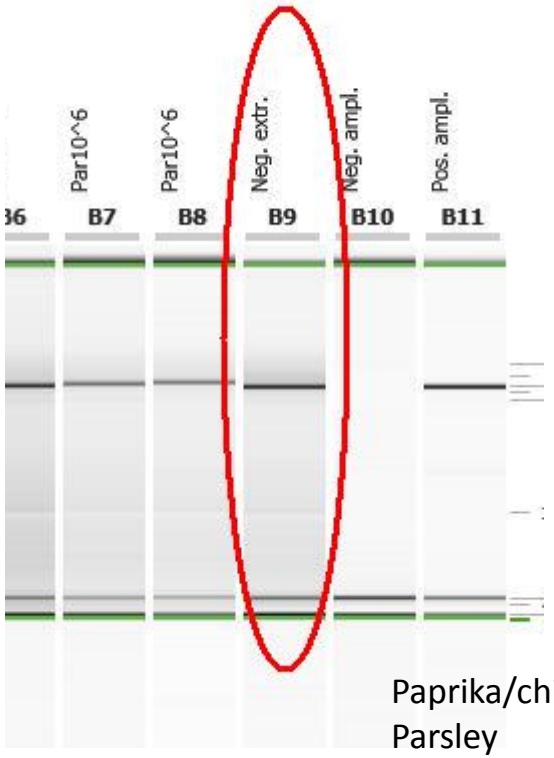
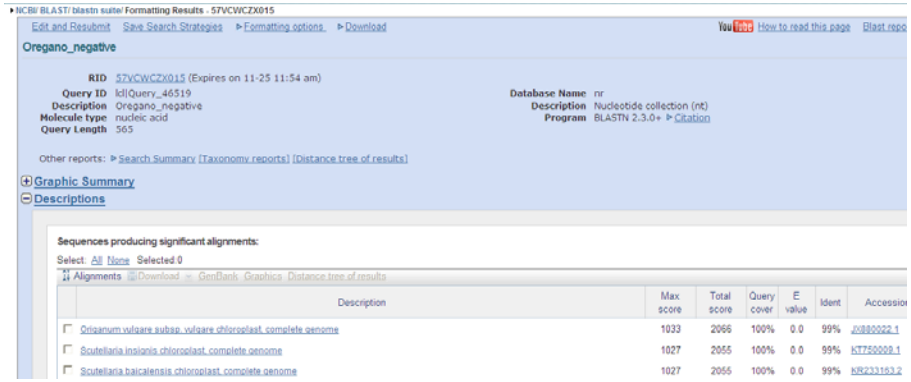
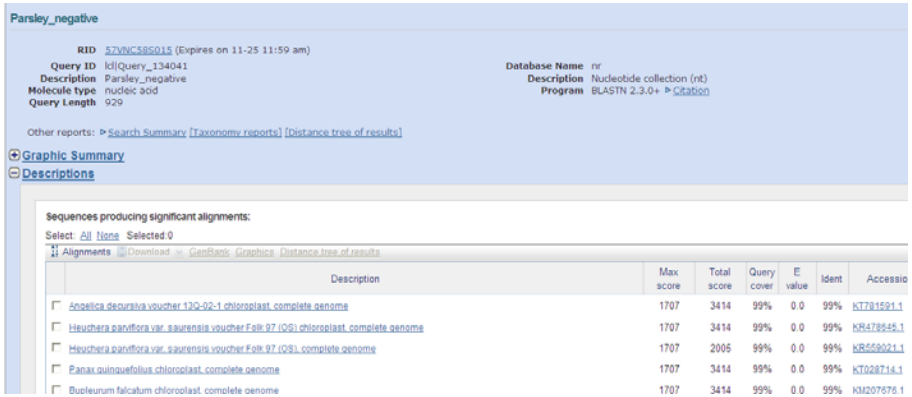


Paprika/chili
Parsley
Oregano





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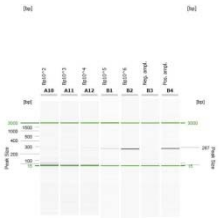
Detection of *Listeria monocytogenes*

DNA extraction
1) CTAB
2) CTAB+PVP

Conventional PCR

Agarose gel electroforesis



•*Listeria monocytogenes* **prfA** gene specific primers conventional PCR

Limit of detection of CTAB and CTAB+PVP extraction protocols combined with conventional PCR targeting L.monocytogenes prfA gene

Matrix	LOD CFU/g	
	CTAB	CTAB+PVP[1)]
Black pepper	$>10^5$ [2)]	10^5
Paprika/chili	10^5	10^5
Oregano	10^5	10^6
Parsley	10^6	10^5
Basil	$>10^6$	$>10^6$
Nutmeg	$>10^6$	10^6
Allspice	$>10^6$	$>10^6$
Cinnamon	$>10^6$	$>10^6$

•One extraction was performed for each matrix
•Two of four tests were positive for this contamination level

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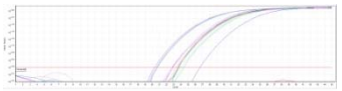
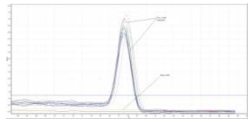
Detection of *Listeria monocytogenes*

•*Listeria monocytogenes* **prfA** gene specific primers SYBR®Green real time PCR

DNA extraction → SYBR®Green real time PCR → Ct data → Melting point analysis



Limit of detection and mean Ct values at level of contamination 10^6 CFU/g of CTAB and CTAB+PVP extraction protocols combined with SYBR Green real-time PCR targeting *L.monocytogenes prfA* gene.

Matrix	CTAB		CTAB+PVP	
	LOD CFU/g	Ct at 10^6 CFU/g	LOD CFU/g	Ct at 10^6 CFU/g
Black pepper	10^5	26.65	10^4	25.00
Paprika/chili	10^3	25.90	10^3	25.35
Oregano	10^5	27.03	10^4	26.70
Parsley	10^5	28.55	10^4	27.20
Basil	10^5	29.57	10^3	20.52
Nutmeg	$>10^6$		10^5	30.38
Allspice	$>10^6$		10^6	30.71
Cinnamon	$>10^6$		10^6	30.10

Two extractions were performed for each matrix with two PCR replicates for each extraction.

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Conclusions

- Use of CTAB method for direct bacterial DNA isolation was successful for all spices and herbs
- Use of PVP in CTAB DNA extraction procedure may improve DNA recovery and PCR performance for some matrices (basil, nutmeg, allspice, cinnamon)
- Additional steps might be needed for difficult matrices like nutmeg, allspice and cinnamon to improve DNA recovery and PCR performance
- The sensitivity 10^3-10^4 CFU/g achieved for *Listeria monocytogenes* is not sufficient for routinely testing
- The sensitivity 10^2-10^4 CFU/g achieved for *Staphylococcus aureus* is sufficient for routinely testing

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Thanks for your attention.
Questions and comments.

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