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

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Date: 01.06.2016.

Location: Berlin, BfR
• 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
CTAB DNA extraction

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

**Amount of starting material:** μgs – several grams

**CTAB lysis:**
+ t°C
+ Proteinase K
+ Rnase
+ β-mercaptopethanol
+ Polyvinylpirrolidone (PVP)

**CTAB precipitation:**
CTAB + low salt conditions

**DNA wash, dry, resuspend**

**Advantages:** low costs, adjustable for a broad type of samples

**Disadvantages:** laborious, time consuming
**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 stable 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.
Detection of *Staphylococcus aureus*

- Each matrix sample (parsley, basil, cinnamon, vanilla) was contaminated with different concentration (10^2 to 10^6 CFU per gram) 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

16S rRNA PCR results *S. aureus*

- *S. aureus* DNA was detectable down to $10^2$ CFU/g in three matrices – parsley, basil, vanilla
- In cinnamon *S. aureus* DNA was detectable down to $10^4$ CFU/g
**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.
Detection of *Listeria monocytogenes*

• Each matrix sample (black pepper, paprika/chili, parsley, oregano, basil, cinnamon, nutmeg, allspice) was contaminated 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
• Conventional and real time PCR detection for prfA gene in two replicates for each extraction was performed.
Detection of Listeria monocytogenes

A universal bacterial primers for 16S rRNA gene

Detection of *Listeria monocytogenes*

*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

<table>
<thead>
<tr>
<th>Matrix</th>
<th>LOD CFU/g</th>
<th>CTAB</th>
<th>CTAB+PVP(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>&gt;10⁵ (^2)</td>
<td>10⁵</td>
<td></td>
</tr>
<tr>
<td>Paprika/chili</td>
<td>10⁵</td>
<td>10⁵</td>
<td></td>
</tr>
<tr>
<td>Oregano</td>
<td>10⁵</td>
<td>10⁶</td>
<td></td>
</tr>
<tr>
<td>Parsley</td>
<td>10⁶</td>
<td>10⁵</td>
<td></td>
</tr>
<tr>
<td>Basil</td>
<td>&gt;10⁶</td>
<td>&gt;10⁶</td>
<td></td>
</tr>
<tr>
<td>Nutmeg</td>
<td>&gt;10⁶</td>
<td>10⁶</td>
<td></td>
</tr>
<tr>
<td>Allspice</td>
<td>&gt;10⁶</td>
<td>10⁶</td>
<td></td>
</tr>
<tr>
<td>Cinnamon</td>
<td>&gt;10⁶</td>
<td>&gt;10⁶</td>
<td></td>
</tr>
</tbody>
</table>

*One extraction was performed for each matrix*

*Two of four tests were positive for this contamination level*
Detection of *Listeria monocytogenes*

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

**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.**

<table>
<thead>
<tr>
<th>Matrix</th>
<th>CTAB LOD CFU/g</th>
<th>CTAB Ct at 10^6 CFU/g</th>
<th>CTAB+PVP LOD CFU/g</th>
<th>CTAB+PVP Ct at 10^6 CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black pepper</td>
<td>10^5</td>
<td>26.65</td>
<td>10^4</td>
<td>25.00</td>
</tr>
<tr>
<td>Paprika/chili</td>
<td>10^3</td>
<td>25.90</td>
<td>10^3</td>
<td>25.35</td>
</tr>
<tr>
<td>Oregano</td>
<td>10^5</td>
<td>27.03</td>
<td>10^4</td>
<td>26.70</td>
</tr>
<tr>
<td>Parsley</td>
<td>10^5</td>
<td>28.55</td>
<td>10^4</td>
<td>27.20</td>
</tr>
<tr>
<td>Basil</td>
<td>10^5</td>
<td>29.57</td>
<td>10^3</td>
<td>20.52</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>&gt;10^6</td>
<td>10^5</td>
<td></td>
<td>30.38</td>
</tr>
<tr>
<td>Allspice</td>
<td>&gt;10^6</td>
<td>10^6</td>
<td></td>
<td>30.71</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>&gt;10^6</td>
<td>10^6</td>
<td></td>
<td>30.10</td>
</tr>
</tbody>
</table>

Two extractions were performed for each matrix with two PCR replicates for each extraction.
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.
Thanks for your attention. Questions and comments.

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