Methods used for the detection of Xylella fastidiosa in the surveillance activities in Germany

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Topic 5.5

Monitoring according to 2015/789/EU

- Inspectors of the Plant Protection Services (PPS) of the federal States sample
  - suspicious plants at places of production and garden centres
  - plants at points of entry
- Inspectors of the PPS trace back notifications of shipments of possibly infected coffea plants and took samples

- Samples were mainly sent to JKI and analysed at my laboratory

- In October 2015 a workshop took place at my laboratory for the detection of *Xylella fastidiosa* with the Laboratories of the federal States

Samples analyzed at the JKI

- *Citrus*, *Nerium oleander* L., *Olea* sp., *Quercus* sp., *Prunus* sp. *Rosmarinus officinalis* L., *Portulaca*, *Vinca minor*, *Veronica* sp., *Vitis*, Coffea sp.
- total number of samples: 168

  Xylella fastidiosa only detected in samples of older plants of *Coffea* sp.

  - with symptoms

  without symptoms

Preparation of the samples

- Midrips and petioles of the leafs (cut in small pieces)

- DNA extractions
  - 0,6 g tissue material: modified CTAB extraction method (Loconsole *et al.*, 2014); up to 5 subsamples
  - 1,5 g tissue material, crashed and resuspended in 0,5 M PB: EasyDNA Kit

    (resususpended tissue was also used for IF-Test)

Tests performed

- **PCR**
- *X. fastidiosa* sspp. according to Minsavage *et al.* (1994)
  - inhibition problems
    - reduced by diluting the extracts (1:20) and adding BSA to mastermixes (0,1 % per reaction)

    ⇨ DNA Extraction using CTAB very reliable and more sensitive than EasyDNA Kit (Invitrogen)
- **IF-Test**
- polyclonal antibody from Loewe
  - working dilution 1:2000

  ⇨ corresponding results with PCR

- *Isolation*
  - *was not successful, due to overgrown by other bacteria*
  - *for positive cultures media used: BCYE and Difco™-Charcoal-Agar*

Workshop for detection of Xf

Aim

- Verifying the detection procedures of the laboratories of the federal States for the monitoring in 2016

Planning

- ✓ with Xf spiked plant extract in (negative coffee extract) was sent to the labs (13 + 1)
  - contamination level: high, medium, low, none,
- ✓ DNA-extraction was performed in the labs according to their routinely used methods for detection of bacteria in plant material
- ✓ PCR to be performed in the lab in Kleinmachnow

Procedure (in Kleinmachnow)

- ✓ people from the Laender-Labs performed the PCR, divided in four groups

Results

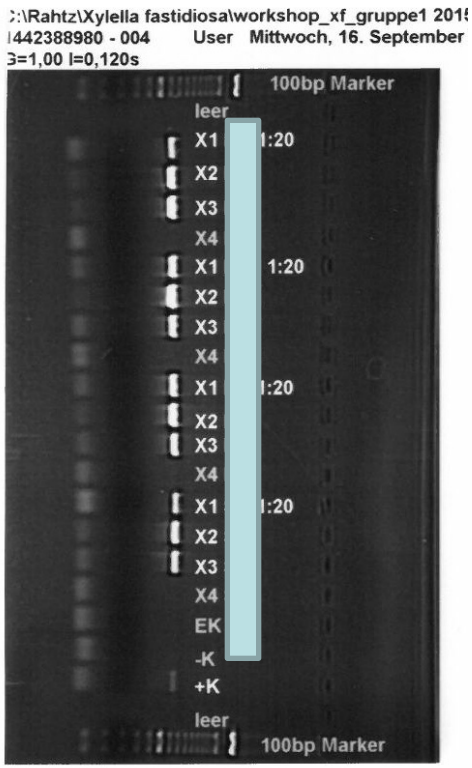
		Labor														
		DNA-extraction method														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
contamination		a	a	b	a	c	a	g	a	d	e	a	d	b	e	f
10ex8	X1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10ex6	X2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10ex4	X3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
none	X4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

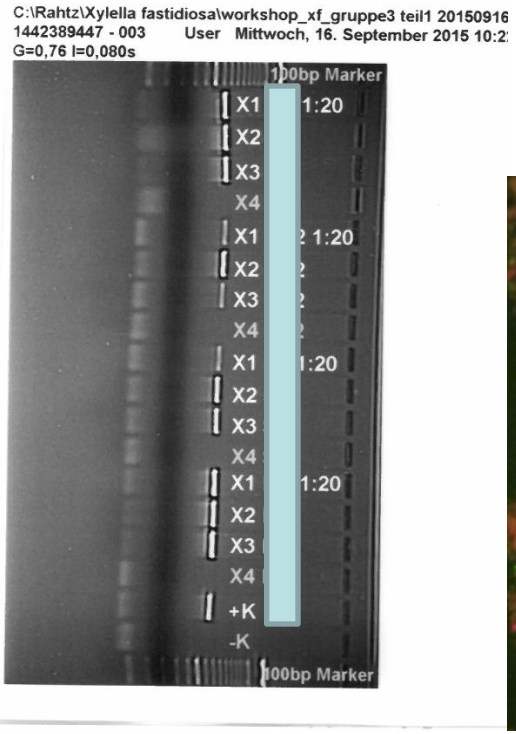
DNA-Extraction methods

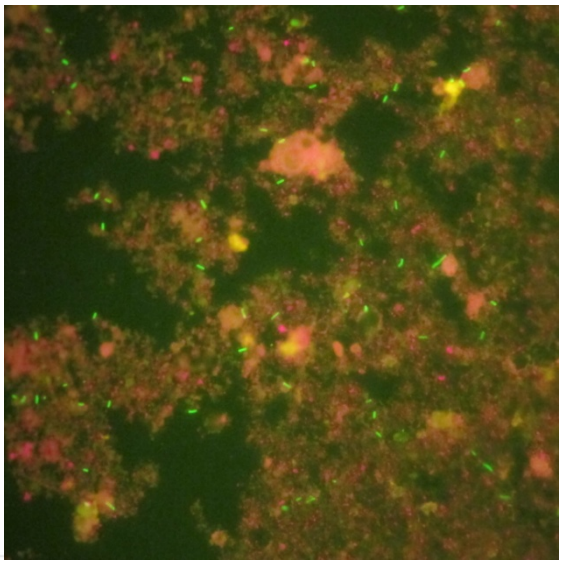
a) QIAmp DNA Mini Kit (Quiagen)
b) Extraktionsautomat QiaCube (QiaGen) DNEasy Plant Mini Kit
c) DNEasy von Quiagen und mit Zymoresearch aufgereinigt
d) GuSCN-Silica
e) Easy DNA Kit (Invitrogen)
f) CTAB
g) KingFisher

- 100 % correct
- slight variation in the DNA extraction quantity depending on the method

Results







Groupwise Gel Electrophoresis of the PCR-Products of the four samples

Flouresing bacteria cells, extracted from natural infected coffee plants; stained with antibody from Loewe (magnification 630x)

Conclusion

- Further evaluation of sample preparation (different hosts) and isolation protocols
- Evaluation of sampling of asymptomatic plants
- Further evaluation of sensitivity of different PCR-protocols
- Further evaluation of sensitivity and specificity of antibody from Loewe (IF-Test)
- Further evaluation of characterization of the different isolates
- Harmonization of the diagnostic methods to be used for the monitoring according to 2015/789/EU and follow up?
  - organizing of a PT for EPPO official laboratories?


Thank you for your attention
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