Survey of Dickeya and Pectobacterium in Poland

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1st Euphresco-II Pectobacterium meeting
21 - 22 November 2013, Jerusalem, Israel
Outline

• General characteristic of bacteria from genera *Dickeya* and *Pectobacterium*

• Characteristics and distribution of *Dickeya* species on seed potato fields in Poland

• Characteristics and distribution of *Pectobacterium* species on seed potato fields in Poland

• Monitoring of *Dickeya* and *Pectobacterium* in Polish waters

• Conclusions
• General characteristic of bacteria from genera *Dickeya* and *Pectobacterium*

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General characteristic of bacteria from genera *Dickeya* and *Pectobacterium*

- Rod-shaped, Gram-negative, motile, facultative anaerobe
- Wide host-range: potato, carrot, tomato, maize, banana, chicory, artichoke and several species of ornamental plants (diffenbachia, hyacinths, dianthus, dahlia, kalanchoe)
- The appearance of the disease symptoms depends on environmental conditions such as temperature, humidity, oxygen availability, condition of the crop etc.
- Cause economically important losses all over the world
Main pathogenicity factors

- Pectate lyases (10 different)
- Pectin lyases
- Pectin methylesterases
- Polygalacturonases
- Cellulases
- Proteases Phospholipases

Predominant role in plant tissue maceration
Other pathogenicity factors

- Siderophores synthesis (to overcome the low iron availability in plant intracellular fluids)
- Motility
- Biosurfactans and biofilm production
- Production of a small signal compounds (QS)
Characteristic of bacteria from genera *Dickeya* and *Pectobacterium*

Symptoms caused by *Dickeya* during vegetation and storage

- **Black leg diseases**
- **Soft rot**
DICKEYA SOLANI A NEW, VERY VIRULENT SPECIES OF DICKEYA
Dickeya strains in Europe

Until 2001

D. dianticola

Toth et. al, 2011; Sarris et. al., 2011; Heuer et al., 2010
Dickeya on potatoes fields in Europe

Dickeya solani in Europe

In 2011

- **D. solani**
- **D. dianticola**

Toth et al., 2011
Pathogenicity of *Dickeya solani* strains

Pathogenicity on tubers of two potato cultivars

![Graph showing pathogenicity of *Dickeya solani* strains on macerated potato tissue (%)](image)

- **Irga**
- **Satina**

**Species and Strains:**
- *D. dianthicola*
- *D. sp.*
- *D. sp., 2005*
- *D. dadantii*
- *D. zeae*
- *P. atrosepticum*
- *P. carotovorum*
- *P. odoriferum*
- **Negative control**

**Pipet tips test**
Genotypic characterization of *Dickeya solani* strains

- Analysis of the sequences of housekeeping genes: *gyrA*, *rpoS*, *gyrA* sequences.

- Whole genomes analysis with the use of repetitive sequences (BOX, REP and ERIC).

- Pulse Field Gel Electrophoresis (PFGE) test.

- All tested strains indicate both identical sequences for the tested housekeeping genes and identical REP-PCR and PFGE patterns.
Current taxonomy of *Dickeya* species

**Genus:** *Dickeya*

**Species:**
- *Dickeya zeae*
- *Dickeya dadantii subsp. dadantii*
- *Dickeya dadantii subsp. dieffenbachiae*
- *Dickeya chrysanthemi*
- *Dickeya paradisiaca*
- *Dickeya dianthicola*

*Dickeya solani*  
(Samson et al., 2005
Brady et al., 2012)

(Wolf et al., 2013)
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Survey of soft rotting bacteria in Poland

- From 100 to 200 samples of symptomatic potato plants or tubers were collected in 1997, 2005, 2009, 2010, 2011 and 2013 by Plant Protection Inspectors.

- About 1500 samples of water were tested in 2010, 2011, 2012 and 2013 for the presence of pectinolytic bacteria (samples were collected by Central Laboratory in Torun).

- Isolates of pectinolytic bacteria were collected, identified and checked for the ability to cause tissue maceration.

The project was performed in cooperation with Governmental Inspection for Plant Protection.
Detection and identification of pectinolytic bacteria isolated from potato plants

Detection and identification of *Pectobacterium* and *Dickeya* spp.


*Dickeya solani* identification

*recA* gene fragment restriction analysis
Method used for fast detection and identification of the presence of *Pectobacterium atrosepticum, Pectobacterium carotovorum/Pectobacterium wasabiae* and *Dickeya* sp. in symptomatic potato plants

1. Plant tissue homogenization

2. Whole DNA isolation

3. Multiplex PCR with primers specific for *Pectobacterium atrosepticum, Pectobacterium carotovorum/Pectobacterium wasabiae* and *Dickeya* sp.

Detection and identification of the pectinolytic bacteria from different species based on the number and size of Multiplex PCR products
Detection and identification of the pectinolytic bacteria from different species based on the number and size of Multiplex PCR products
Detection and identification of pectinolytic bacteria isolated from potato plants

Detection and identification of *Pectobacterium* and *Dickeya* spp.


*Dickeya solani* identification

*recA* gene fragment restriction analysis
Phylogenetic analysis of Dickeya strains based on recA sequences

The analysis was performed using MUSCLE and PhyML tools

Dickeya solani
Molecular method for identification of *Dickeya solani*

1. *In silico* analysis of the *recA* gene sequence of *Dickeya* and *Pectobacterium* strains; analysis of the 735 bp fragment of *recA* sequences (Waleron et al. 2002) but not a short fragment of 481 bp (Parkinson et al. 2009).

2. Identification of the unique restriction site for *XbaI* present only in the sequence of *D. solani recA* gene; 603 bp and 132 bp fragments.

3. Strains from *Dickeya* and *Pectobacterium* were tested by *recA* PCR RFLP with *XbaI*.


Application of three step procedure for fast detection and identification of the presence of *Dickeya solani*:

1. Isolation of the DNA from bacterial cells

2. PCR with primers based on the *pel* genes sequences and specific for *Dickeya* sp. (Nassar et al. 1996)

3. PCR amplification of *recA* gene fragment (Waleron et al. 2002) and digestion of the obtained product with *XbaI* restriction endonucleases

Identification of the *D. solani* is based on the presence of unique *XbaI* restriction site in the *D. solani* *recA* gene
Development of the test for fast identification of the *Dickeya solani* strains

Isolation of pectinolytic bacteria from plant tissue and PCR from cells lysate

PCR with primers based on the *pel* genes sequences and specific for *Dickeya* sp. (Nassar et al. 1996)

PCR amplification of *recA* gene fragment (Waleron et al. 2002) and digestion of the obtained product with *XbaI* restriction endonucleases

Identification of the *D. solani* is based on the presence of unique *XbaI* restriction site in the *D. solani recA* gene

(Waleron et al., 2013)
### Pectinolytic bacteria detected in on seed potato plantation in Poland

<table>
<thead>
<tr>
<th>Samples</th>
<th>Year</th>
<th>2005</th>
<th>2009</th>
<th>2011</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of samples</td>
<td></td>
<td>146</td>
<td>188</td>
<td>176</td>
<td>231</td>
</tr>
<tr>
<td>No of samples with <em>Pectobacterium carotovorum</em> subsp. <em>carotovorum</em> and <em>Pectobacterium wasabiae</em> detected</td>
<td></td>
<td>38</td>
<td>35</td>
<td>94</td>
<td>32</td>
</tr>
<tr>
<td>Number of samples with <em>Pectobacterium atrosepticum</em> detected</td>
<td></td>
<td>77</td>
<td>75</td>
<td>72</td>
<td>30</td>
</tr>
<tr>
<td>Number of samples with <em>Dickeya solani</em> detected</td>
<td></td>
<td>1</td>
<td>6</td>
<td>27</td>
<td>8</td>
</tr>
<tr>
<td>% of sample in which bacteria form the genus <em>Dickeya</em> were detected</td>
<td></td>
<td>0.6%</td>
<td>3%</td>
<td>15%</td>
<td>3,4%</td>
</tr>
</tbody>
</table>
Occurrence of *Dickeya* spp. strains on potato plants in Poland
Dickeya solani strains detected in Poland in 2013

Geographical distribution

Source of the isolated strains

- Stem: 75%
- Tuber: 12%
- Weed: 13%
Outline

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• Conclusions
Pectinolytic bacteria from the genus Pectobacterium was classified to 5 subspecies on the basis of 16S rDNA

Genus: *Pectobacterium*
Species: *Pectobacterium carotovorum*
Subspecies: *Pectobacterium carotovorum* subsp. *atrosepticum*
*Pectobacterium carotovorum* subsp. *betavascularum*
*Pectobacterium carotovorum* subsp. *carotovorum*
*Pectobacterium carotovorum* subsp. *odoriferum*
*Pectobacterium carotovorum* subsp. *wasabaie*

(Kwon et al. 1997, Hauben et al. 1998)
Elevation of three subspecies of *Pectobacterium carotovorum* to species level: 120 phenotypic features, DNA:DNA hybridisation and 16SrDNA sequence

Genus: *Pectobacterium*
Species: *Pectobacterium atrosepticum*
*Pectobacterium carotovorum*
*Pectobacterium carotovorum* subsp. *carotovorum*
*Pectobacterium carotovorum* subsp. *odoriferum*
*Pectobacterium betavascularum*
*Pectobacterium wasabaie*  
(Gardan et al., 2003)

*Pectobacterium carotovorum* subsp. *brasiliensis*  
(Duarte et al., 2004)
Reclassification of *P. c. subsp. carotovorum* strain SCC3193 to *P. wasabiae*

Nykeri et al. 2012 Revised phylogeny of the model soft rot phytopathogen *Pectobacterium wasabiae* SCC3193 former *Pectobacterium carotovorum* subsp. *carotovorum*

Synteny of *Pectobacterium wasabiae* and *Pectobacterium carotovorum* genomes
Waleron et al., 2002. **Genotyping of bacteria belonging to the former Erwinia genus by PCR-RFLP analysis of recA gene fragment**

- Pcc SCC3193 (P. wasabiae SCC3193) belongs to group 3 recA PCR-RFLP, (with application of four restriction endonucleases),
- all *P. wasabiae* from horseradish belong to group 23 recA PCR-RFLP,
- differences only in pattern of one restriction endonuclease,

All Pcc from group 3 recA PCR-RFLP are very virulent,

In 2009 the Padlock probe for Pcc group 3 recA PCR-RFLP was designed,

2012 - vPcc (de Haan et al. 2008) has the same sequence of *recA* as *P. wasabiae* SCC3193

Sławiak et al., 2013. **Multiplex detection and identification of bacterial pathogens causing potato blackleg and soft rot in Europe, using padlock probes**

Waleron et al., 2013. **Occurrence of Pectobacterium wasabiae in potato field samples**
Identification of *P. wasabiae* strains in Europe and reclasification of strains earlier classified as *P. carotovorum* susp. *carotovorum*

recA gene sequence from IFB collection of 150 strains of *Pectobacterium* was analysed

*P. wasabiae TS* (isolated from horseradish)

*P. wasabiae SCC3193*

*P. wasabiae, from potatoes*

*P. atrosepticum*

*P. carotovorum*

(Waleron *et al.*, 2013; Sławiak *et al.* 2013)
All of *Pectobacterium wasabiae* strains isolated from potato plants and tubers are highly pathogenic on potato.

Some of potato *Pectobacterium wasabiae* are able to grow at 37°C and are able to grow on medium with 5% NaCl.

*Pectobacterium wasabiae* Type Strain and other strains isolated from horseradish are not able to grow at 37°C and are not able to grow on medium with 5% NaCl.
Three species/subspecies of *Pectobacterium* are detected in *potrato* plantation in Poland

*Pectobacterium atrosepticum*
*Pectobacterium carotovorum*
*Pectobacterium carotovorum subsp. carotovorum*
*Pectobacterium carotovorum subsp. odoriferum*
*Pectobacterium carotovorum subsp. brasiliensis*
*Pectobacterium betavascularum*
*Pectobacterium wasabaie*
Characteristics and distribution of *Pectobacterium* species and subspecies on potato fields in Europe

*P. wasabiae* in Europe

also:
USA, Canada, Peru, South Africa, New Zealand

(Waleron et. al, 2013)
Distribution of *Pectobacterium* species and subspecies on potato fields in Poland

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<td>77</td>
</tr>
<tr>
<td>Number of samples with <em>Dickeya</em> spp. detected</td>
<td>1</td>
</tr>
<tr>
<td>% of sample in which bacteria form the genus Pcc and Pwa were detected</td>
<td>0.6%</td>
</tr>
</tbody>
</table>
Source of *Pectobacterium* spp. detected in 2013

*Pectobacterium carotovorum /
Pectobacterium wasabiae* (about 15%)

*Pectobacterium atrosepticum*
Geographic distribution of *Pectobacterium spp.* on the territory of Poland in 2013

**Pectobacterium carotovorum** /**Pectobacterium wasabiae**

**Pectobacterium atrosepticum**
Contribution of specific species in detected pectinolytic bacteria

1996

- *Pectobacterium carotovorum/Pectobacterium wasabiae*: 54%
- *Pectobacterium atrosepticum*: 46%
- *Dickeya sp.*: 2%

2005

- *Pectobacterium carotovorum/Pectobacterium wasabiae*: 17%
- *Pectobacterium atrosepticum*: 81%
- *Dickeya sp.*: 2%
Contribution of specific species in detected pectinolytic bacteria

2011

- Pectobacterium carotovorum/Pectobacterium wasabiae: 49%
- Pectobacterium atrosepticum: 37%
- Dickeya sp.: 14%

2013

- Pectobacterium carotovorum/Pectobacterium wasabiae: 46%
- Pectobacterium atrosepticum: 43%
- Dickeya sp.: 11%
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Pectinolytic bacteria detection in water samples

- Water from 20 samples
- Filtration
- Membrane + H₂O
- 20 ml
- 100 μl
- CVP (incubation at 28°C and 37°C)
- Multiplex PCR
- PCR
### Dickeya sp. strains isolated from Polish water

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of water samples tested</td>
<td>1794</td>
<td>2874</td>
<td>1827</td>
<td>1402</td>
</tr>
<tr>
<td>Number of water samples with strains of <em>Dickeya</em> spp.</td>
<td>6</td>
<td>19</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Number of water samples with strains of <em>Pectobacterium wasabiae</em></td>
<td>no tested</td>
<td>no tested</td>
<td>no tested</td>
<td>0</td>
</tr>
</tbody>
</table>

*some of strains identified as *Dickeya zeae*

**P. atrosepticum** and *P. carotovorum subsp. carotovorum* were isolated respectively from 4 and 5 water samples
Conclusions

Bacteria from genera *Dickeya* and *Pectobacterium* are widely distributed in Poland and cause economic losses.

The first *D. solani* strains were isolated from symptomatic potato plant in 2001 but now spread in several regions of Poland.

Simple test for identification of *D. solani* was developed.

*Pectobacterium wasabiae* were isolated from seed potato plantation in Poland.

Bacteria from genera *Dickeya* and *Pectobacterium*, but not *D. solani* and *P. wasabiae*, were isolated from Polish waters
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Agata Motyka, PhD student,
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Dr. Sylwia Jafra
Dr. Wojciech Śledź
Dr. Robert Czajkowski
This work was supported by:

Polish National Science Centre, OPUS project, 2011-2014

Human Capital, National Cohesion Strategy, Life Sciences and Mathematics Interdisciplinary Doctoral Studies; grants for PhD students: Marta Potrykus and Małgorzata Golanowska
Thank you for your attention!
Molecular characterization of new *Dickeya solani* strains with the use of BOX PCR
Genotypic characterization of the *Dickeya solani* strains

Pulse Field Gel Electrophoresis pattern of tested *Dickeya* strains

<table>
<thead>
<tr>
<th>Strains of other <em>Dickeya</em> species</th>
<th>Polish isolates</th>
<th>Finish isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. CH 10 *D. dadantii* TS
2. CH 16 *D. dadantii* 3937
3. CH 55 *D. chrysanthemi* TS
4. CH 103 *D. dianthicola* TS
5. CH 117 *D. paradisiaca* TS
6. CH 99 *D. solani*, Poland 2005
7. CH 158 *D. solani*, Poland 2009
8. CH 296 *D. solani*, Poland 2011
9. CH 300 *D. solani*, Poland 2011
10. CH 304 *D. solani*, Poland 2011
11. CH 309 *D. solani*, Poland 2011
12. CH 311 *D. solani*, Poland 2013
13. CH 313 *D. solani*, Poland 2013
14. CH 318 *D. solani*, Poland 2013
15. CH 129 *D. solani*, France
16. CH 132 *D. solani*, Izrael
17. CH 133 *D. solani*, Scotland
18. CH 134 *D. solani*, Finland
19. CH 225 *D. solani*, Finland
20. CH 226 *D. solani*, Finland
21. CH 123 *D. solani*, The Netherlands
22. Marker *S. cerevisiae* (BIO-RAD)