Survey of Dickeya and Pectobacterium in Poland

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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

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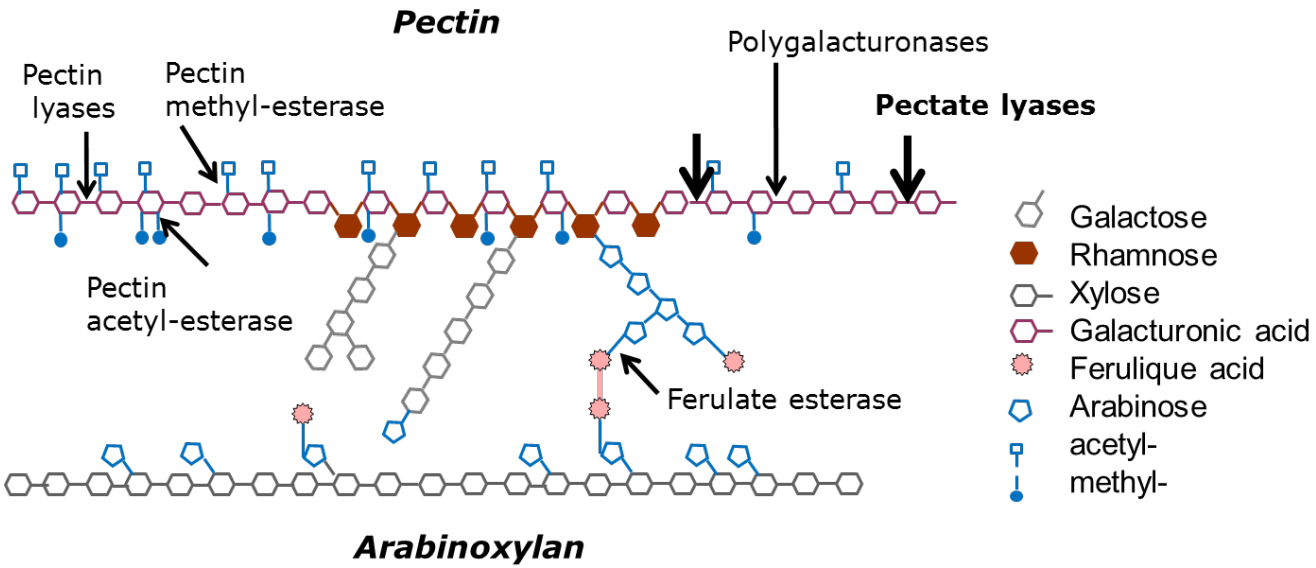
General characteristic of bacteria from genera *Dickeya* and *Pectobacterium*

- Rod-shaped, Gram-negative, motile, facultative anaerobe
- Wide host-range: potato, carrot, tomato, maize, banana, chicory, artichoke and several species of ornamental plants (diffenbachiae, hiacints, 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 biofim 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

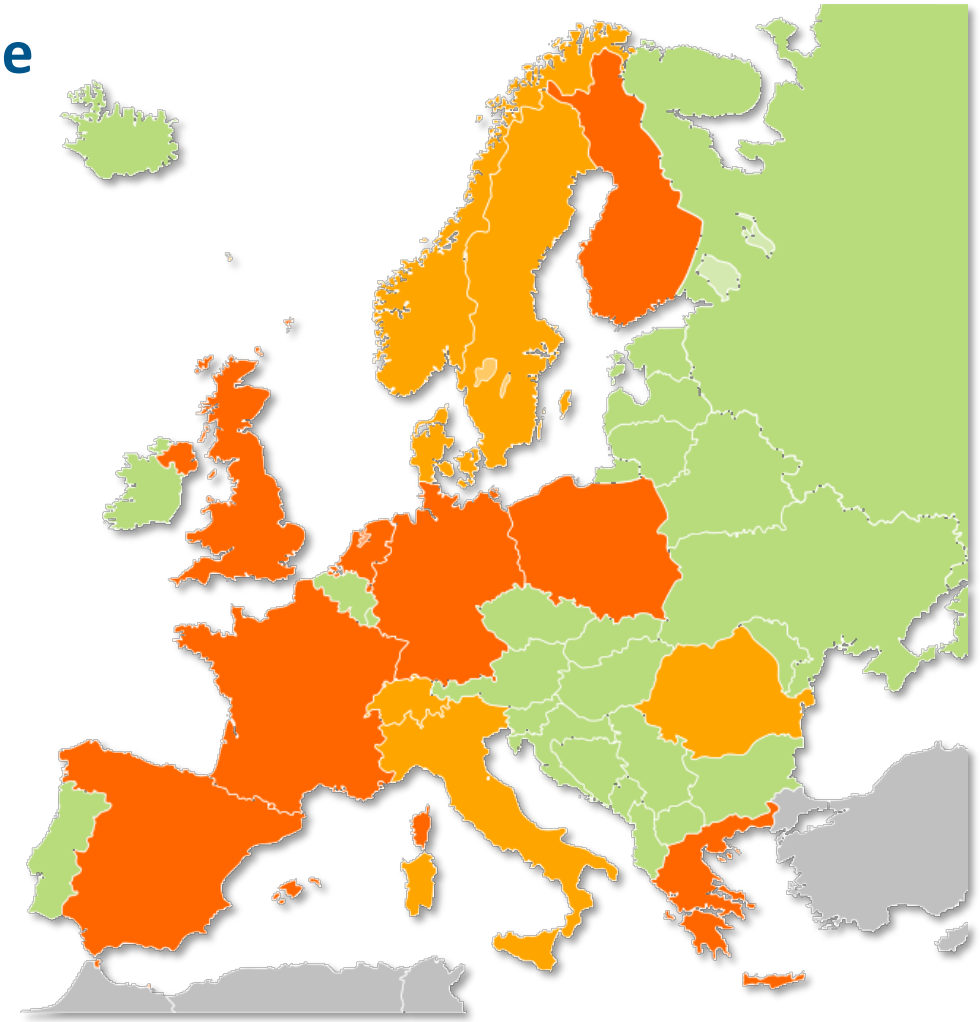
● ***D. dianticola***

Toth *et. al*, 2011; Sarris *et. al*., 2011; Heuer *et al*., 2010

Dickeya on potatoes fields in Europe

Dickeya solani in Europe

In 2011

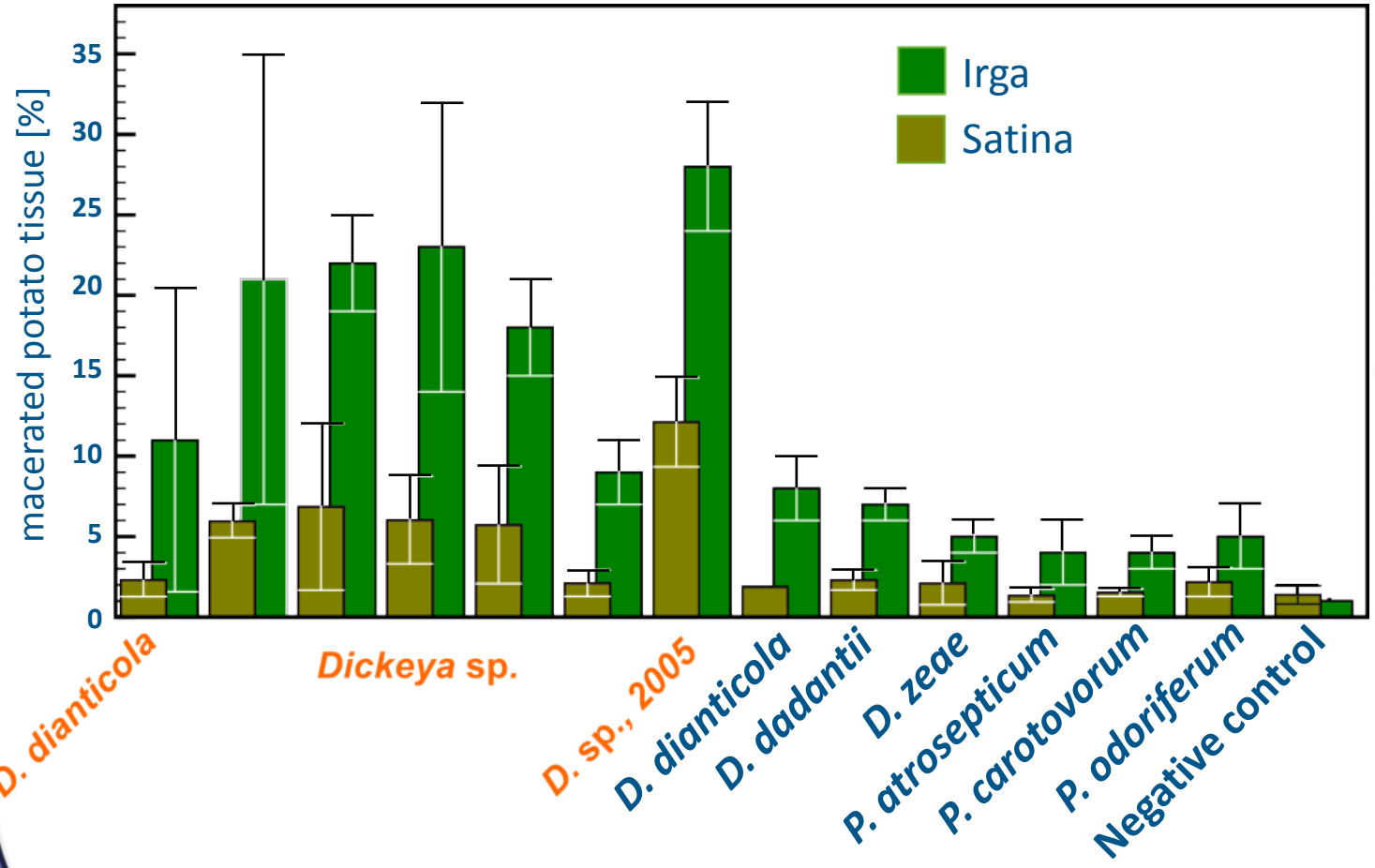


- ***D. solani***
- ***D. dianticola***

Toth et al., 2011

Pathogenicity of *Dickeya solani* strains

Pathogenicity on tubers of two potato cultivars



Pipet tips test

Genotypic characterization of *Dickeya solani* strains

- Analysis of the sequences of houskeeping genes: *gyrA*, *rpoS*, *gyrA* sequences.
- Whole genomes analysis with the use of repetetive 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

(Samson et al., 2005
Brady et al., 2012)

Dickeya solani (Wolf et al., 2013)

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Survey of soft rotting bacteria in Poland

- ✓ From 100 two 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 (sample were collected by Central Laboratory in Torun)
- ✓ Isolates of pectinolytic bacteria were collected, identified and check for the ability 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.

Detection and identification by Multiplex PCR with species/subspecies specific PCR primers (Frechon et al. 1998, Kang et al. 2003, et al. 1996, Laurila *et al.* 2010)

***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

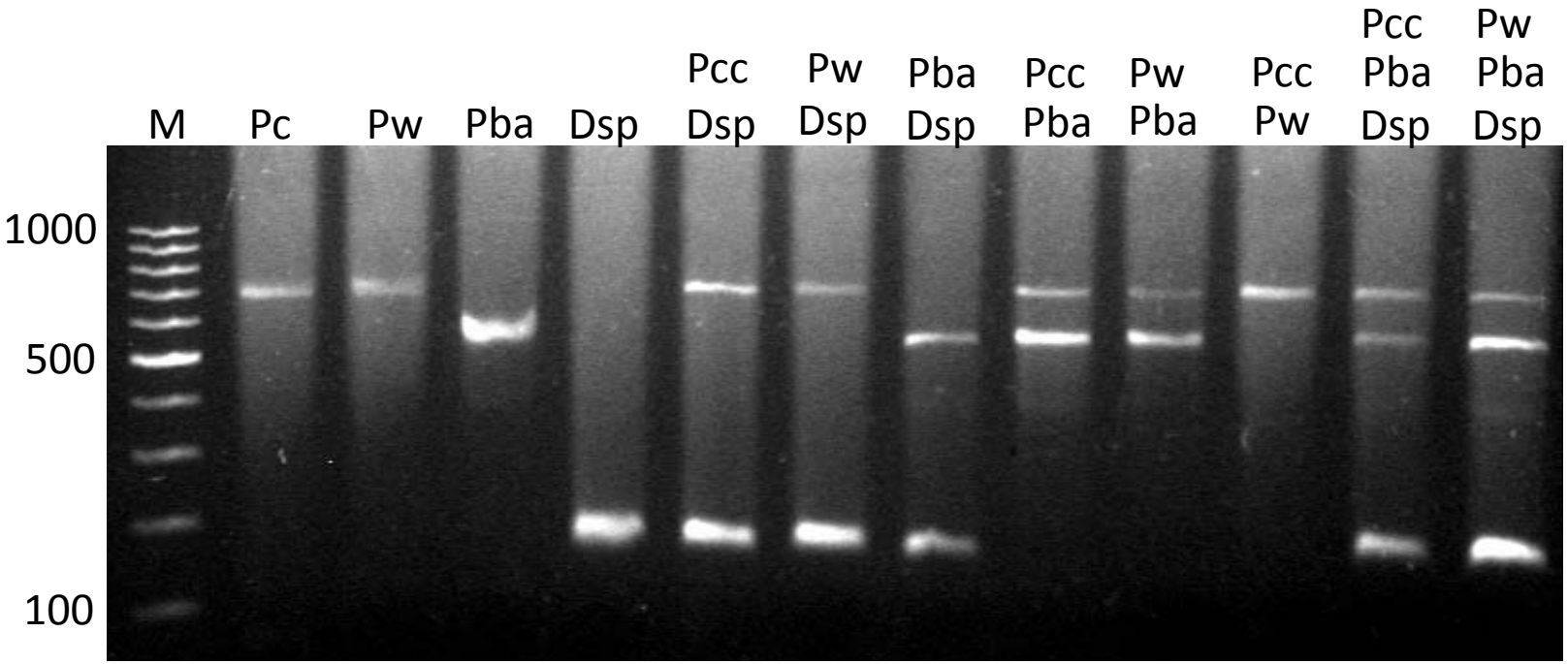
2. Whole DNA isolation

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

Characteristics and distribution of *Pectobacterium* species and subspecies on potato fields in Europe

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

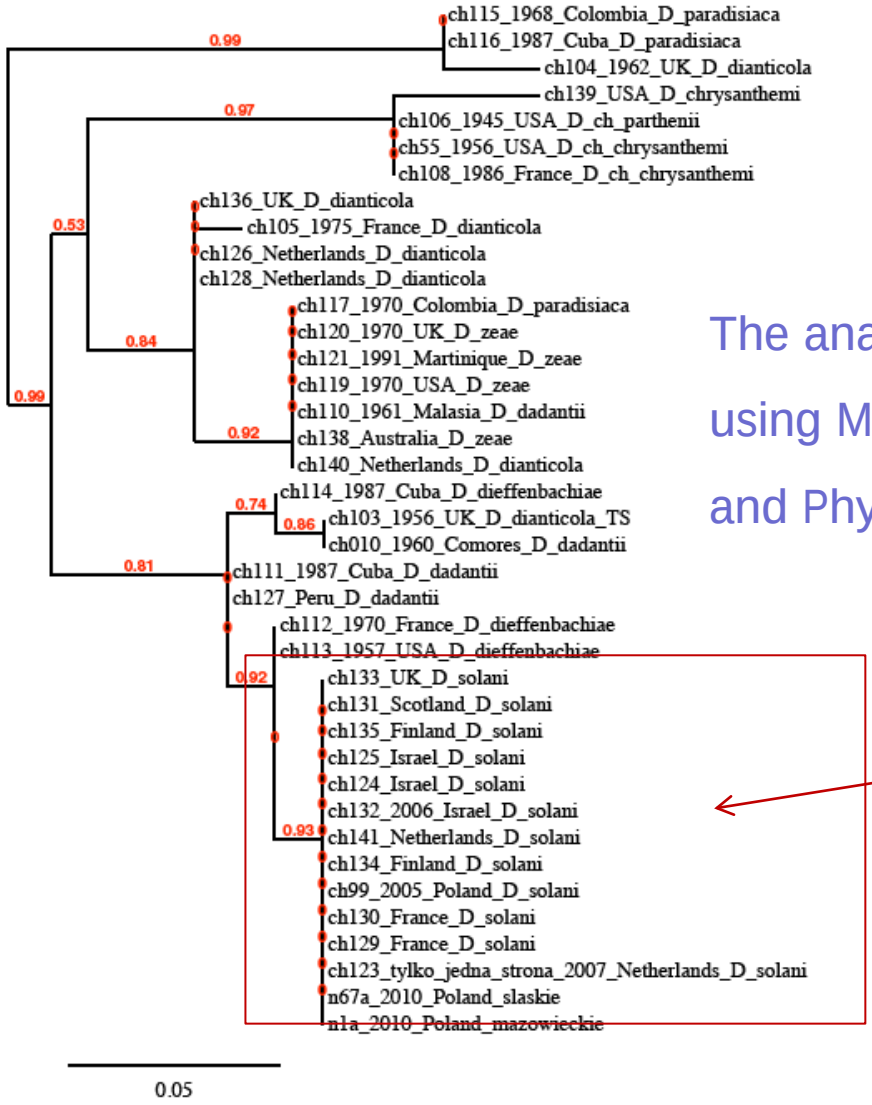
Detection and identification of *Pectobacterium* and *Dickeya* spp.

Detection and identification by Multiplex PCR with species/subspecies specific PCR primers (DeBoer and Ward 1984, Nassar et al. 1996, Laurila *et al.* 2010)

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.*

46 strains of *Dickeya:* *D. solani (19), D. zeae (7), D. dadantii (7), D. chrysanthemi (5), D. paradisiaca (3), D. dianthicola (5).*

19 strains of *Pectobacterium:* *P. atrosepticum (3), P. c.* subsp. *carotovorum (5), P. betavascularum (2), P. wasabiae (3),* P. subsp. *odoriferum (2), P.* subsp. *brasiliensis (2), P. cacticidum (2).*

Molecular method for identification of *Dickeya solani*

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

Molecular method for identification of *Dickeya solani*

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)

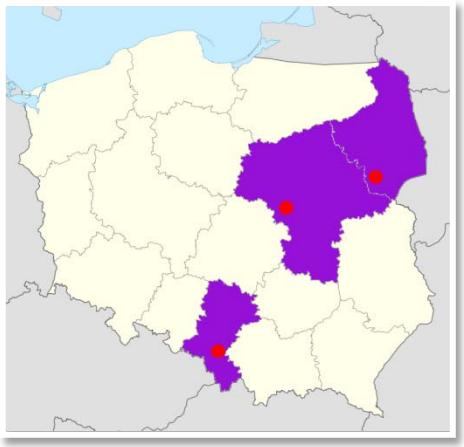
Pectinolytic bacteria detected in on seed potato plantation in Poland

Samples	Year			
	2005	2009	2011	2013
No of samples	146	188	176	231
No of samples with *Pectobacterium carotovorum* subsp. *carotovorum and Pectobacterium wasabiae* detected	38	35	94	32
Number of samples with *Pectobacterium atrosepticum* detected	77	75	72	30
Number of samples with *Dickeya solani* detected	1	6	27	8
% of sample in which bacteria form the genus *Dickeya* were detected	0.6%	3%	15%	3,4%

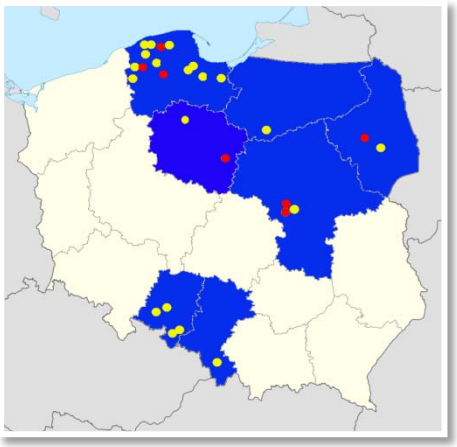
Occurrence of *Dickeya* spp. strains on potato plants in Poland

2005

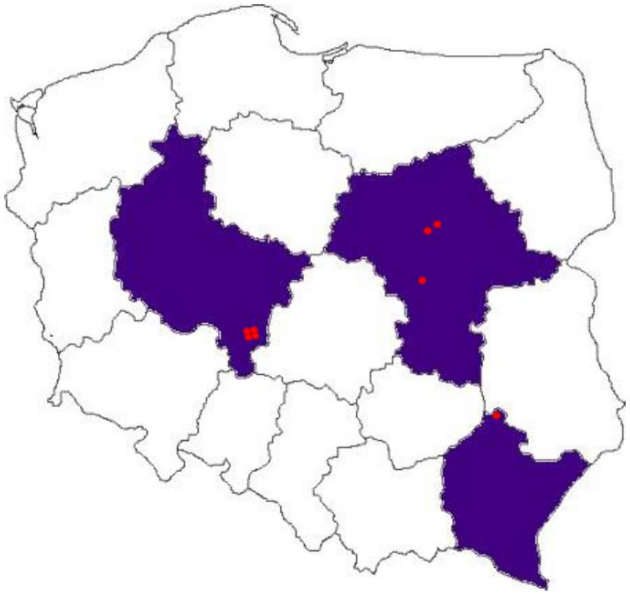
2009



2010



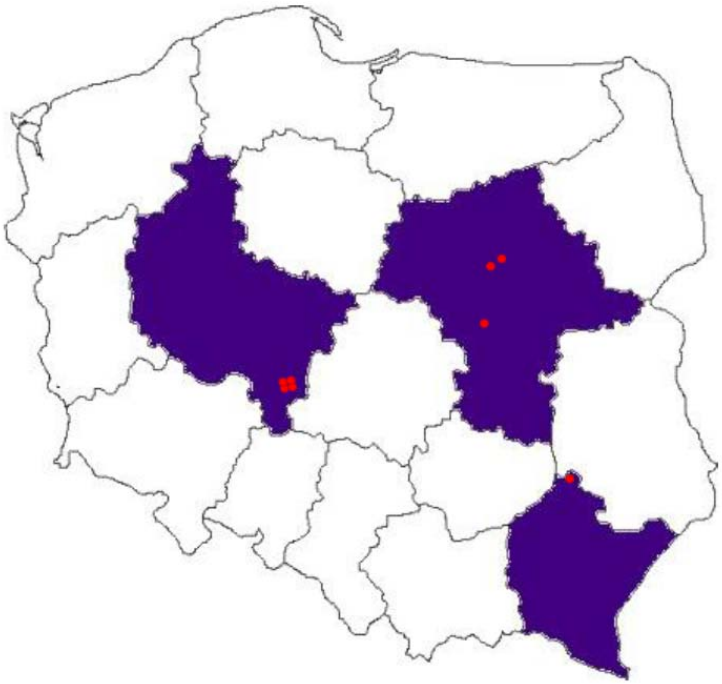
2011



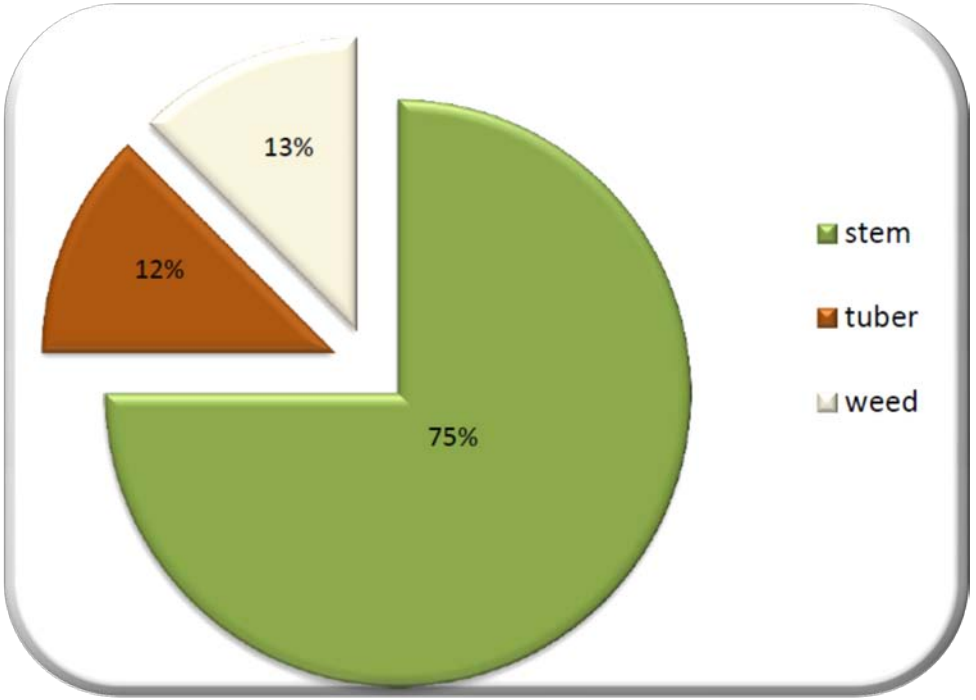
2013

Dickeya solani strains detected in Poland in2013

Geographical distribution



Source of the isolated strains



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MWB

Pectinolytic bacteria from the genus Pectobacterium was clasified 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)

Reclasification of P. c. subsp. *carotovorum* strain SCC3193 to *P. wasabiae*

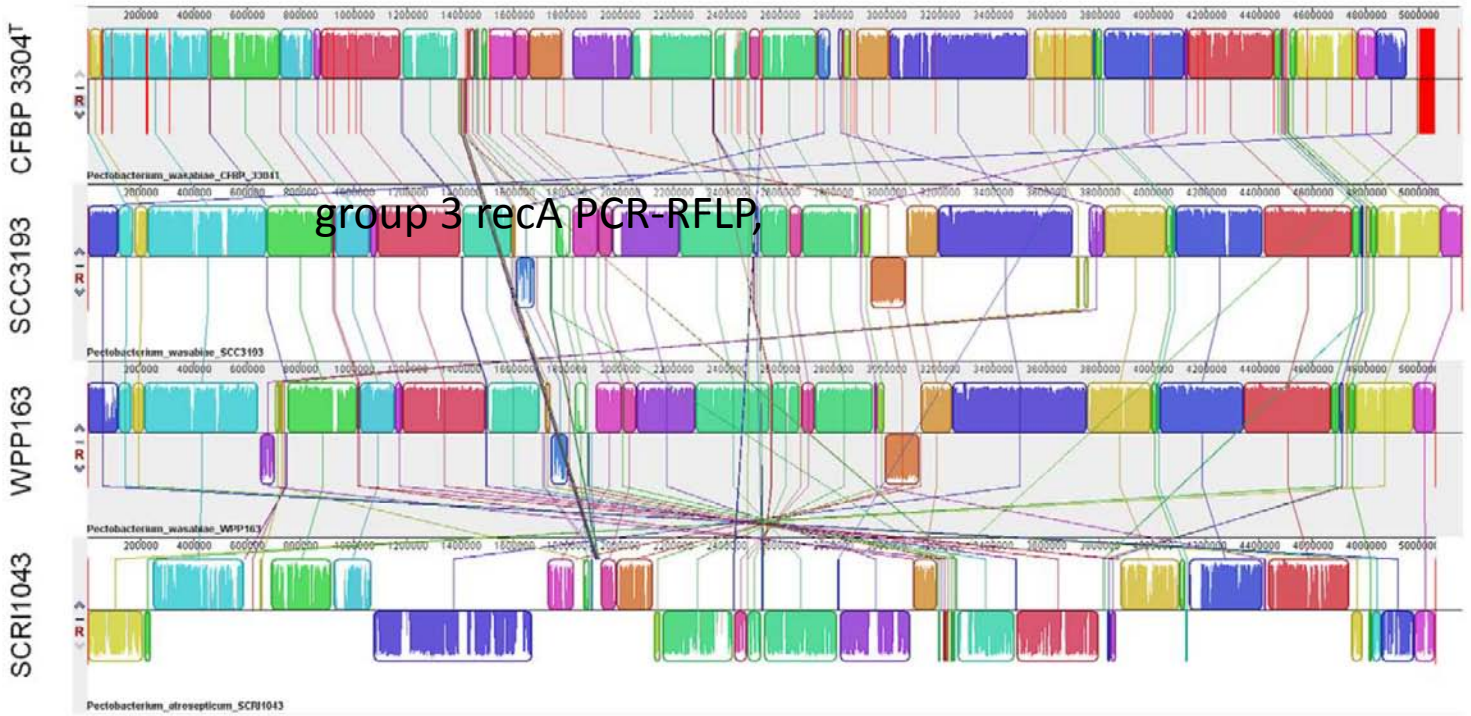
Nykeri et al. 2012 Revised phylogeny of the model soft rot phytopathogen *Pectobacterium wasabiae* SCC3193 former *Pectobacterium carotovorum* subsp. *carotovorum*

***P. wasabaie* TS**

P. wasabaie* former *P. carotovorum

P. wasabaie

P. c. atrosepticum



Synteny of *Pectobacterium wasabiae* and *Pectobacterium carotovorum* genomes

Pectobacterium wasabie story

Waleron et al., 2002. **Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of *rec*A 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**

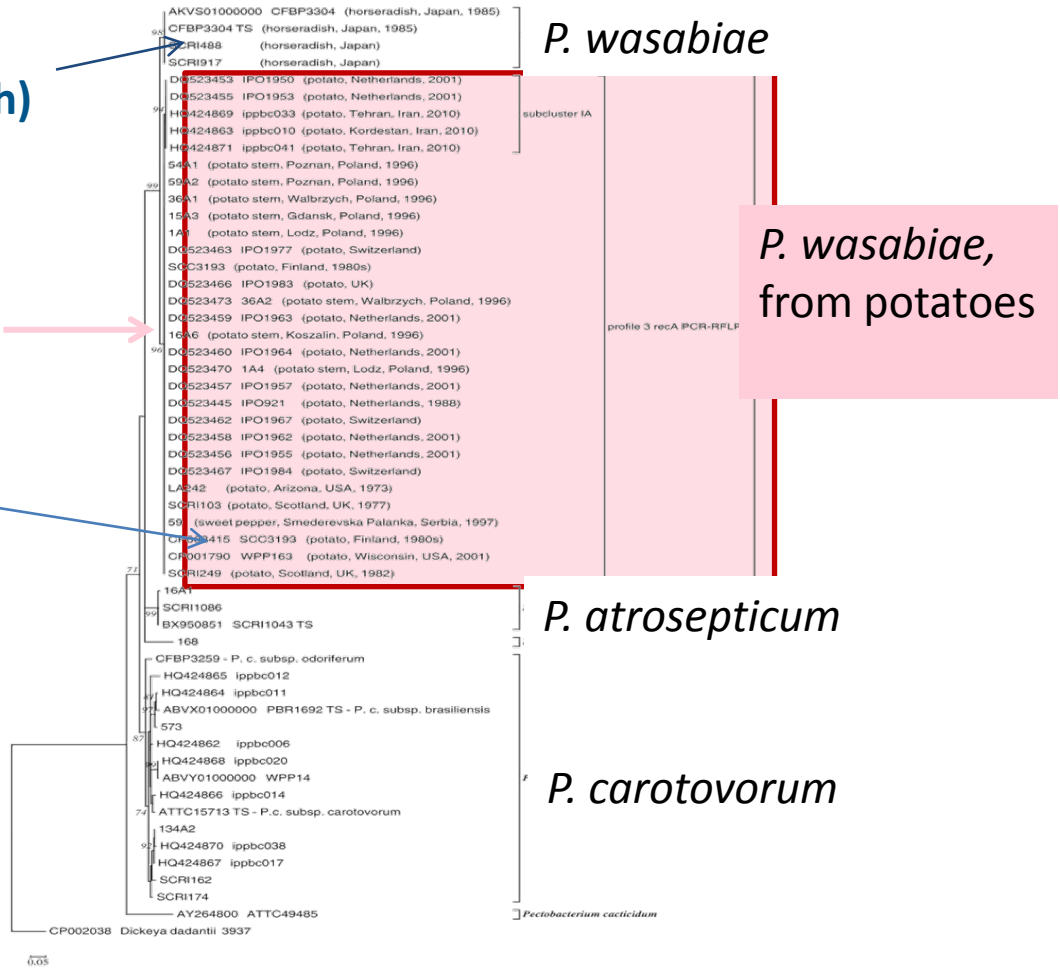
Waleron et al., 2013. **Occurrence of *Pectobacterium wasabiae* in potato field samples**

Identification of *P. wasabiae strains in Europe and reclasification of strains earlier clasified as P. carotovorum susp. carotovorum*

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

***P. wasabie* TS**
(isolated from horseradish)

***P. wasabiae* SCC3193**



(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 37^0C 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 37^0C and are not able to grow on medium with 5% NaCl.**

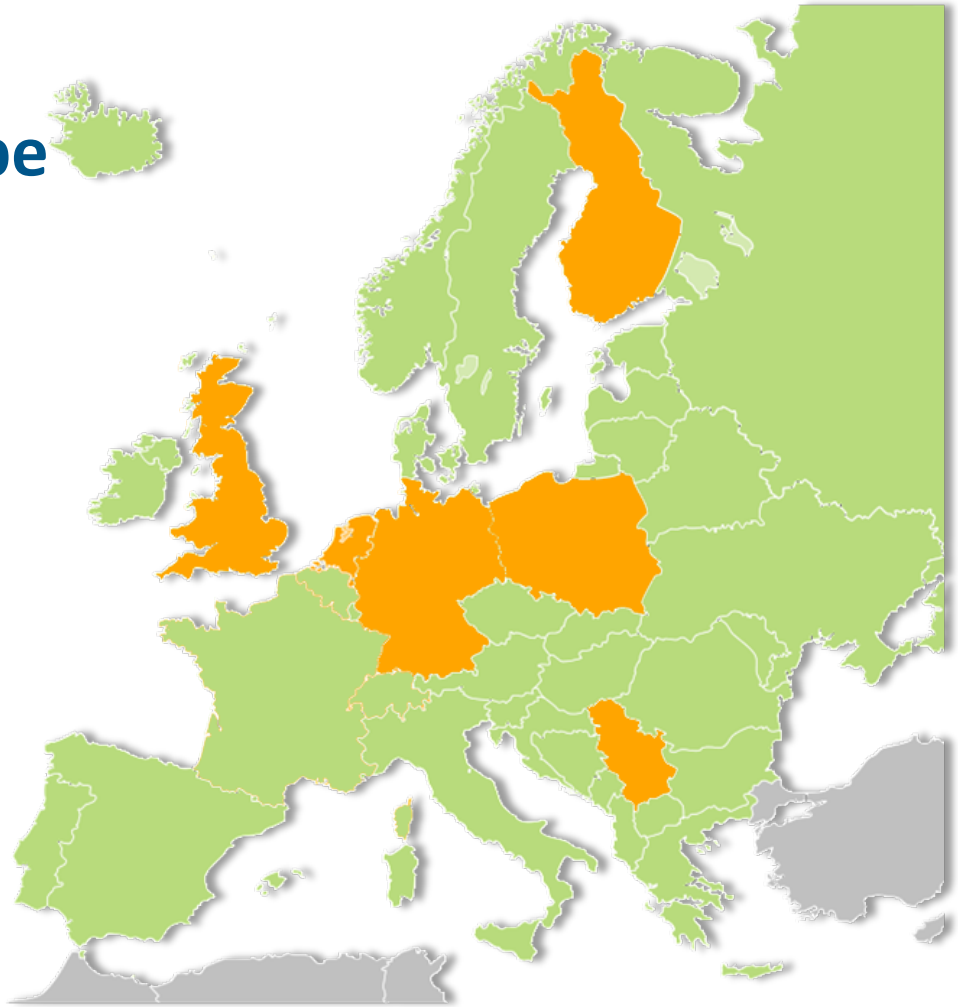
Pectobacterium species and subspecies

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



P. wasabiae

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

(Waleron *et. al*, 2013)

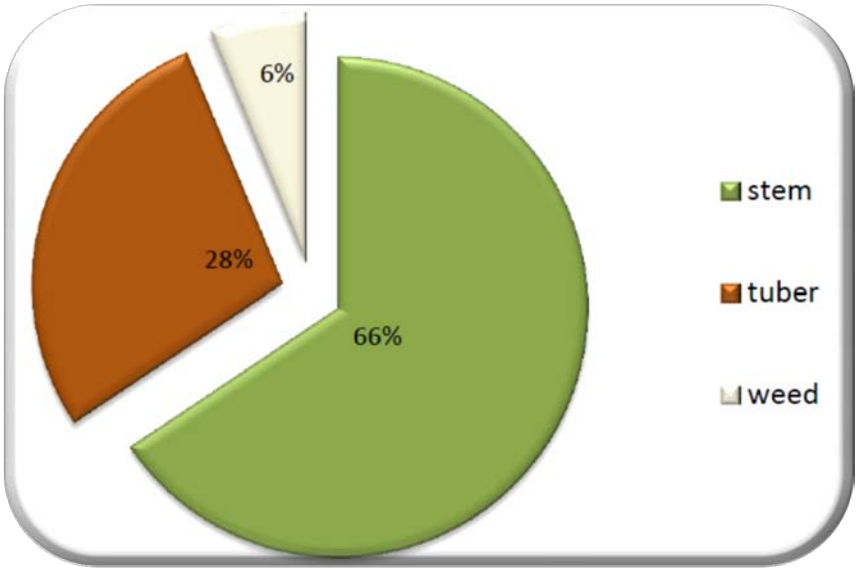
Distribution of *Pectobacterium* species and subspecies on potato fields in Poland

Pectinolytic bacteria detected in on seed potato plantation in Poland

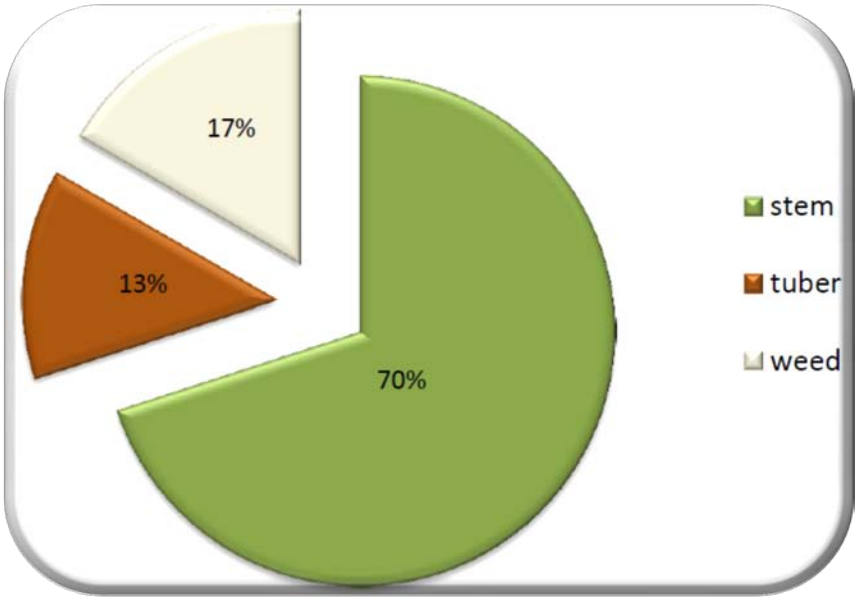
Samples	Year			
	2005	2009	2011	2013
No of samples	**146**	**188**	**176**	**231**
No of samples with *Pectobacterium carotovorum* subsp. *carotovorum* and *Pectobacterium wasabiae* detected	**38**	**35**	**94**	**32**
Number of samples with *Pectobacterium atrosepticum* detected	**77**	**75**	**72**	**30**
Number of samples with *Dickeya* spp. detected	1	6	27	8
% of sample in which bacteria form the genus Pcc and Pwa were detected	**0.6%**	**3%**	**15%**	**14%**

Source of *Pectobacterium spp.* detected in 2013

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

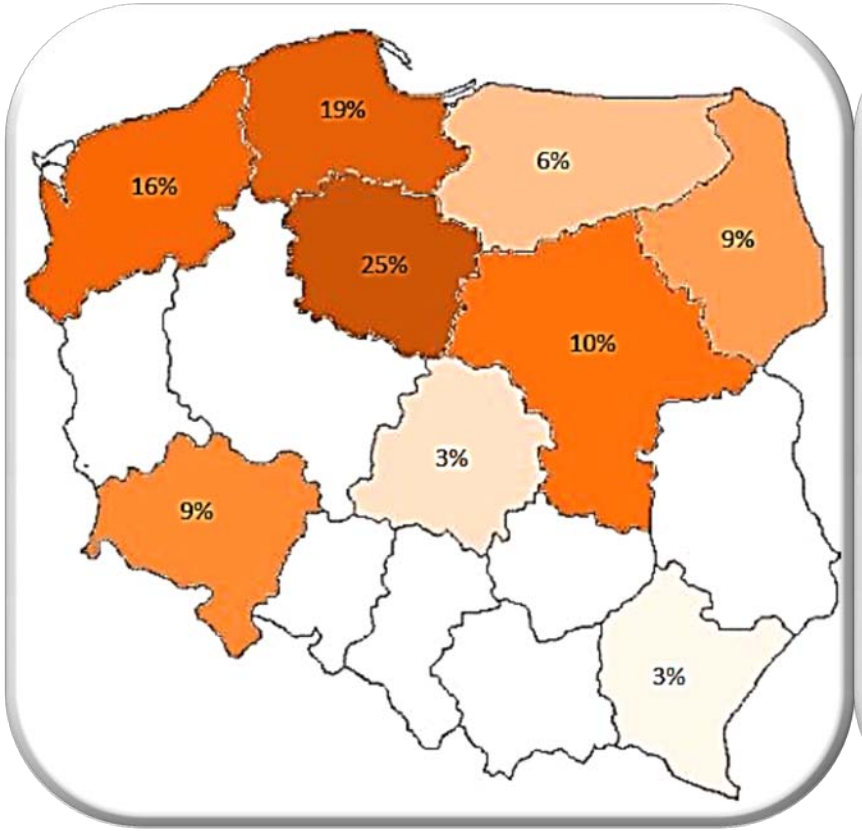


Pectobacterium atrosepticum

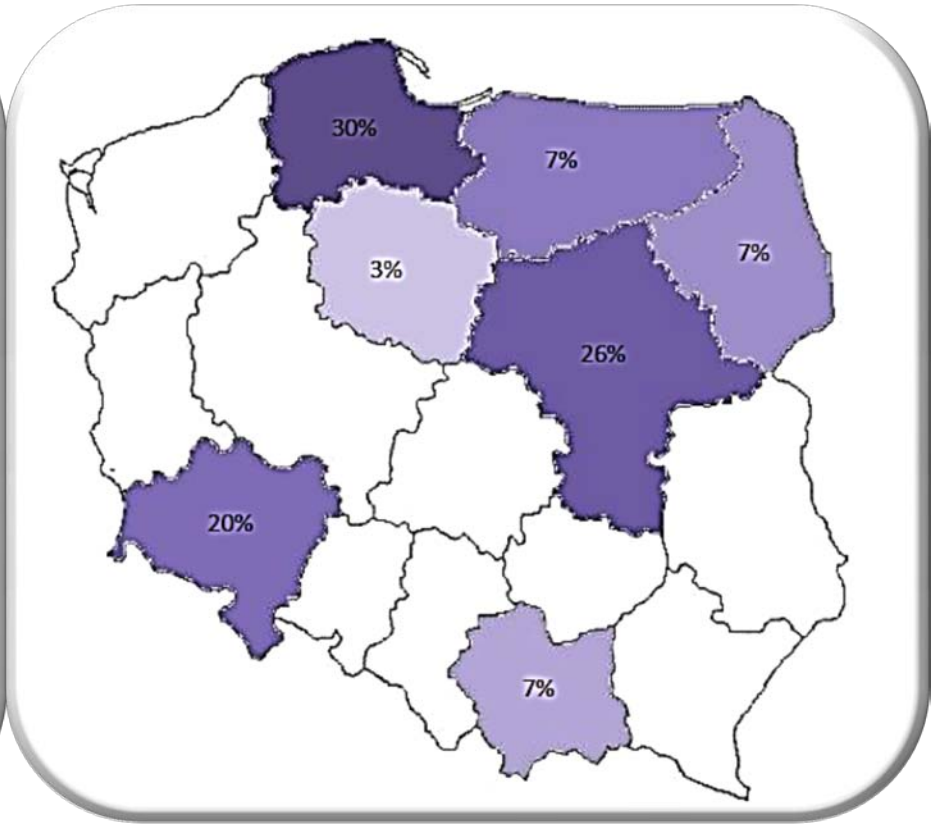


Geographic distribution of *Pectobacterium spp.* on the territory of Poland in 2013

Pectobacterium carotovorum /Pectobacterium wasabiae

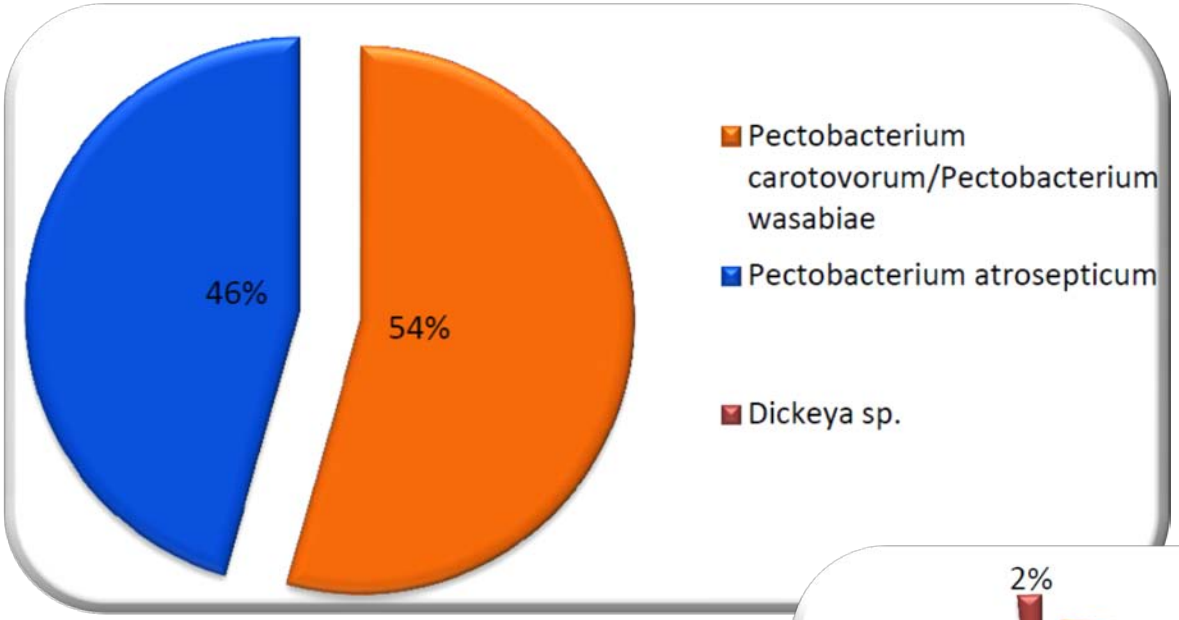


Pectobacterium atrosepticum

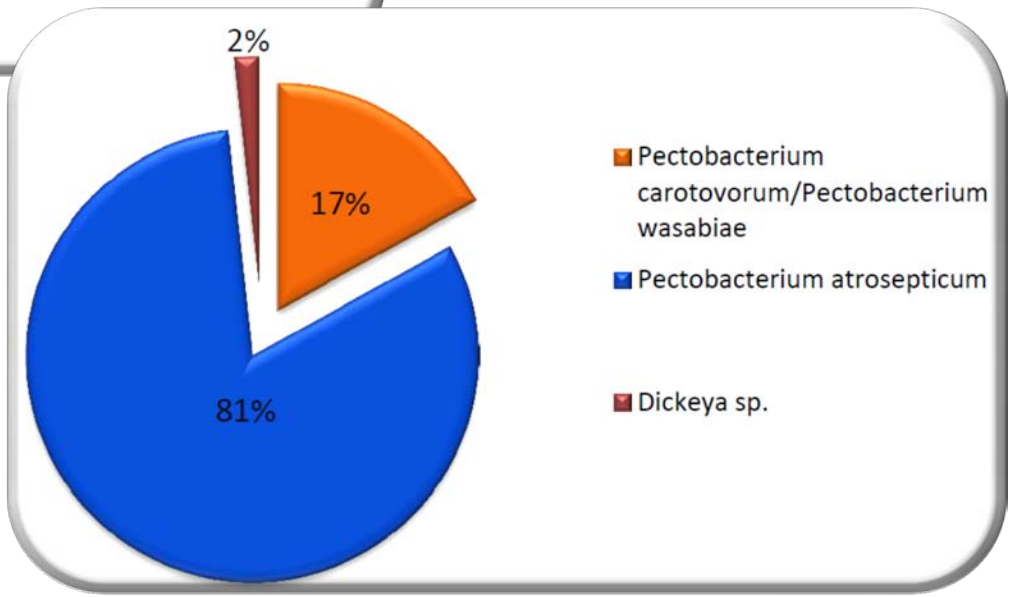


Contribution of specific species in detected pectinolytic bacteria

1996

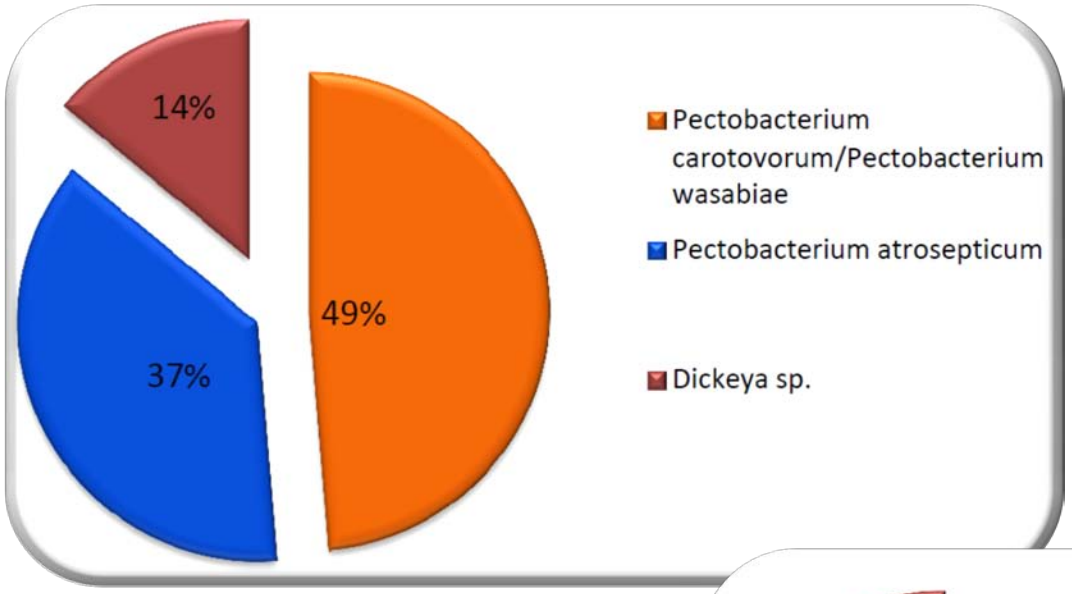


2005

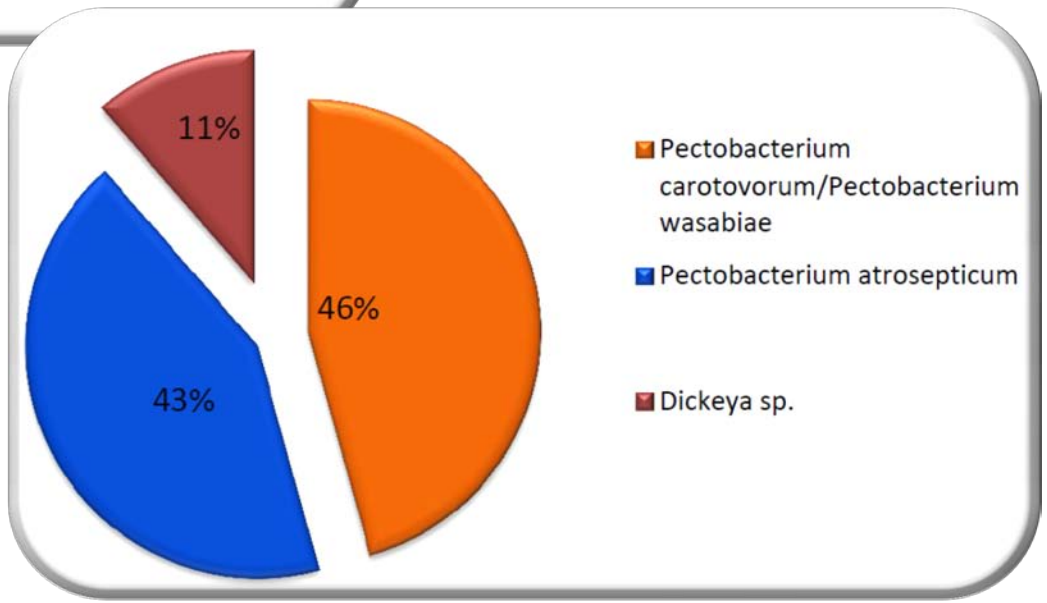


Contribution of specific species in detected pectinolytic bacteria

2011



2013



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Pectinolytic bacteria detection in water samples

Water from 20 samples

20 ml

Filtration

Membrane + H_20

Multiplex PCR

PCR

100 µl

CVP
(incubation at 28^0C and 37^0C)

UNIVERSITY OF GDANSK

Dickeya sp. strains isolated from Polish water

	2010	2011	2012	2013
Number of water samples tested	1794	2874	1827	1402
Number of water samples with strains of *Dickeya* spp.	6	19	4	6
Number of water samples with strains of *Pectobacterium wasabiae*	no tested	no tested	no tested	0

*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 w*ere 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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Dr. Sylwia Jafra
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Dr. Robert Czajkowski

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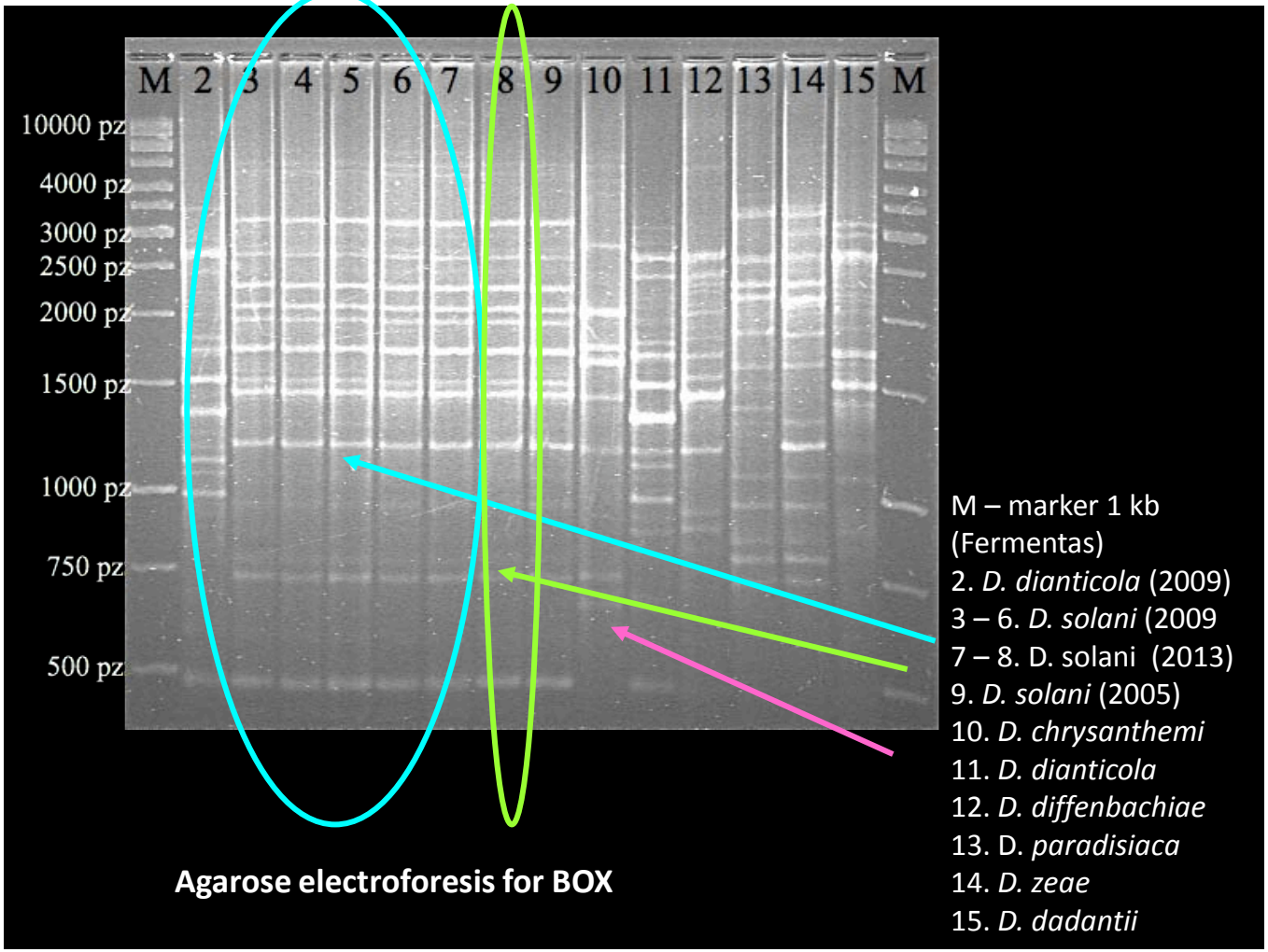
Thank you for your attention !

Genotypic characterization of the *Dickeya solani* strains

Molecular characterization of new *Dickeya* solani strains with the use of BOX PCR



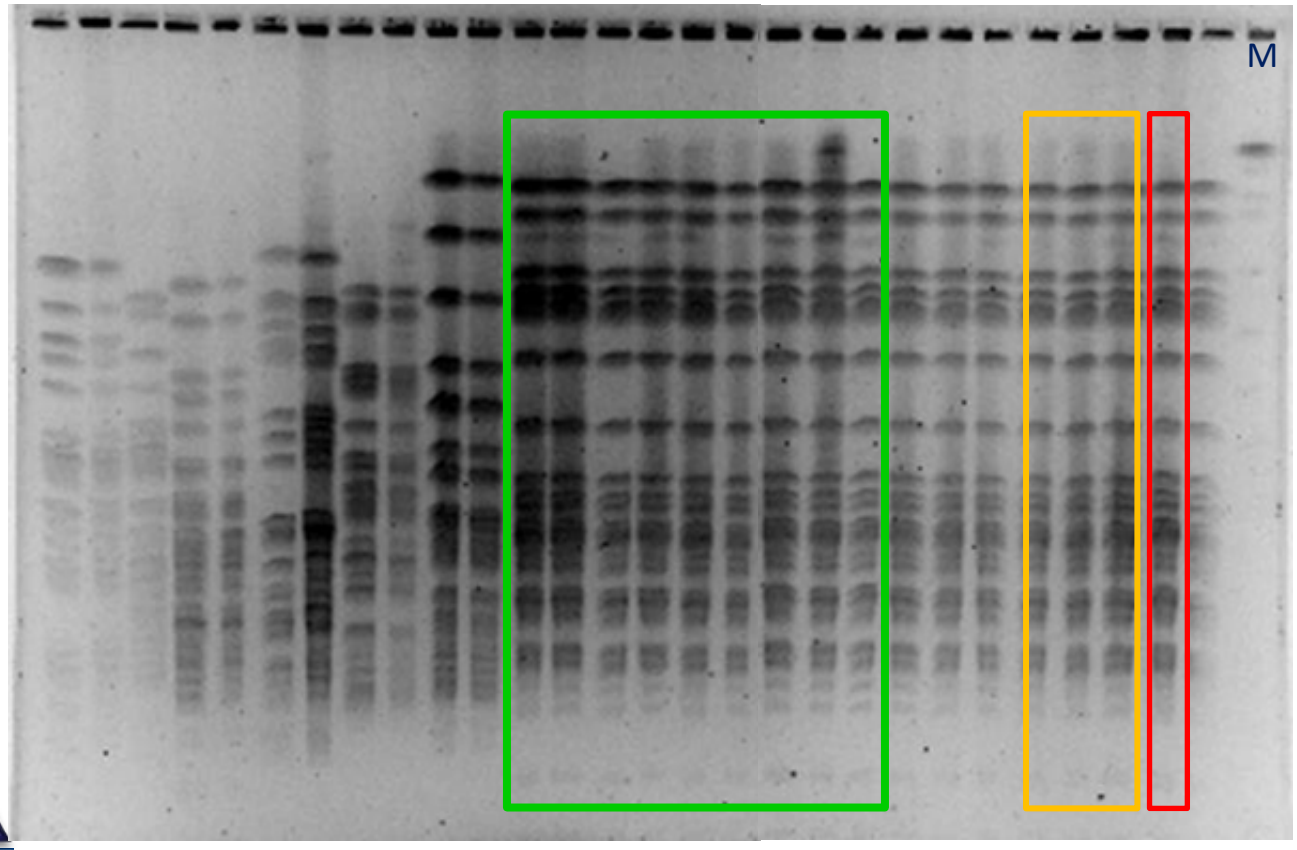
Agarose electroforesis for BOX

Genotypic characterization of the *Dickeya solani* strains

Pulse Field Gel Electrophpresis pattern of tested *Dickeya* strains

Strains of other *Dickeya* species **Polish isolates** **Finish isolates**

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



1, 2. CH 10 *D. dadantii* TS
3. CH 16 *D. dadantii* 3937
4, 5. CH 55 *D. chrysanthemi* TS
6, 7. CH 103 *D. dianthicola* TS
8, 9. CH 117 *D. paradisiaca* TS
10, 11. CH 119 *D. zeae* TS
12. CH 99 *D. solani*, Poland 2005
13. CH 158 *D. solani*, Poland 2009
14. CH 296 *D. solani*, Poland 2011
15. CH 300 *D. solani*, Poland 2011
16. CH 304 *D. solani*, Poland 2011
17. CH 309 *D. solani*, Poland 2011
18. CH 311 *D. solani*, Poland 2013
19. CH 313 *D. solani*, Poland 2013
20. CH 318 *D. solani*, Poland 2013
21. CH 129 *D. solani*, France
22. CH 132 *D. solani*, Izrael
23. CH 133 *D. solani*, Scotland
24. CH 134 *D. solani*, Finland
25. CH 225 *D. solani*, Finland
26. CH 226 *D. solani*, Finland
27. CH 123 *D. solani*, The Netherlands
28. CH 221 *D. solani*, Germany
29. Marker *S. cerevisiae* (BIO-RAD)