



UNIVERSITY OF GDANSK



# Population studies of *Pectobacterium wasabiae* strains isolated from potato plants in Poland

Sabina Zoledowska, Agata Motyka, Dominika Zukowska, Wojciech Sledz, Ewa Lojkowska

Intercollegiate Faculty of Biotechnology  
Department of Biotechnology  
Laboratory of Plant Protection and Biotechnology

# Potato blackleg and soft rot

Potato blackleg:  
Results in stunting, wilting, chlorosis of leaves,  
necrosis of tissues and no emergence.  
Occurs in field.



Potato soft rot:  
Whole tuber maceration, while the 'skin' of potato  
is intact. Occurs in storage.

Caused by pectinolytic bacteria from genera:  
*Dickeya* spp. and *Pectobacterium* sp.



# Plant pathogenic bacterium: *Pectobacterium wasabiae*

- Isolated from horseradish in Japan in 1987 (Goto and Mashumoto, 1987)
- In 2010 described for the first time as a putative pathogen of potato in New Zealand (Pitman et al., 2010)
- In 2012 the strain of *Pectobacterium carotovorum* subsp. *carotovorum* SCC 3193 (model strain for molecular study of Pcc) was reclassified to *Pectobacterium wasabiae* (Nykyri et al., 2012)
- From 2012 there have been published few reports confirming *P. wasabiae* presence in Europe (Nabhan et al., 2012, Waleron et al., 2013, Pasanen et al., 2013)

# Aims of the project

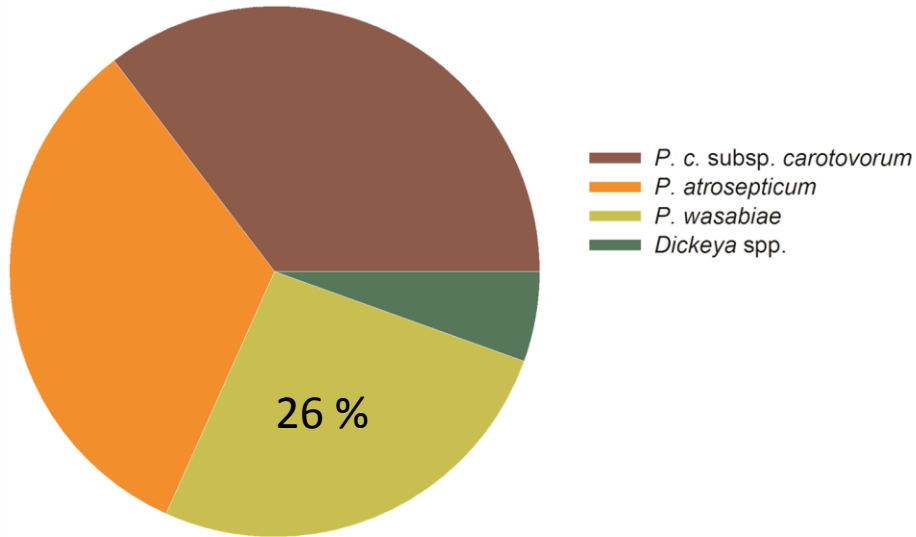
- Monitoring of *Pectobacterium wasabiae* on seed potato fields in Poland
- Characterization of obtained Pwa strains:
  - Genotypic
  - Phenotypic
- Population structure analysis

# MONITORING

*Pectobacterium wasabiae* isolated from potato field samples obtained in years 2013 and 2014

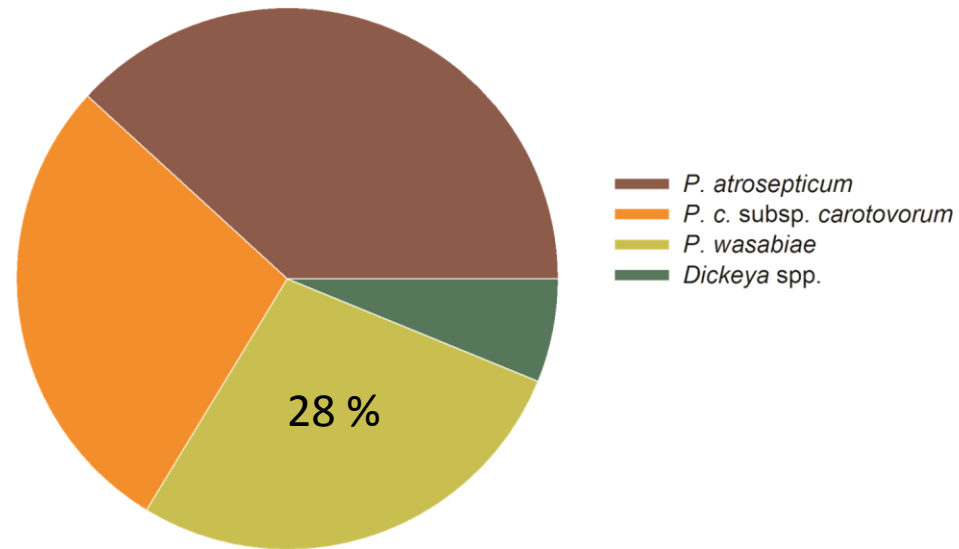
# Structure of pectinolytic bacteria population— years 2013 and 2014

Monitoring 2013



From 185 potato seed plants samples  
40 *P. wasabiae* strains were isolated.

Monitoring 2014



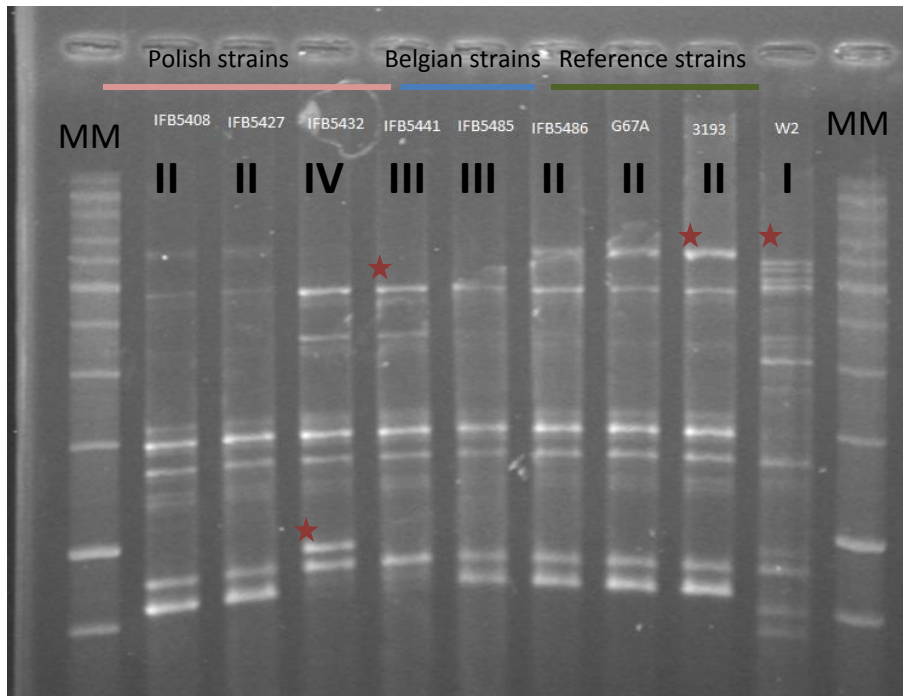
From 262 potato seed plants samples  
42 *P. wasabiae* strains were isolated.

# GENOTYPIC CHARACTERIZATION

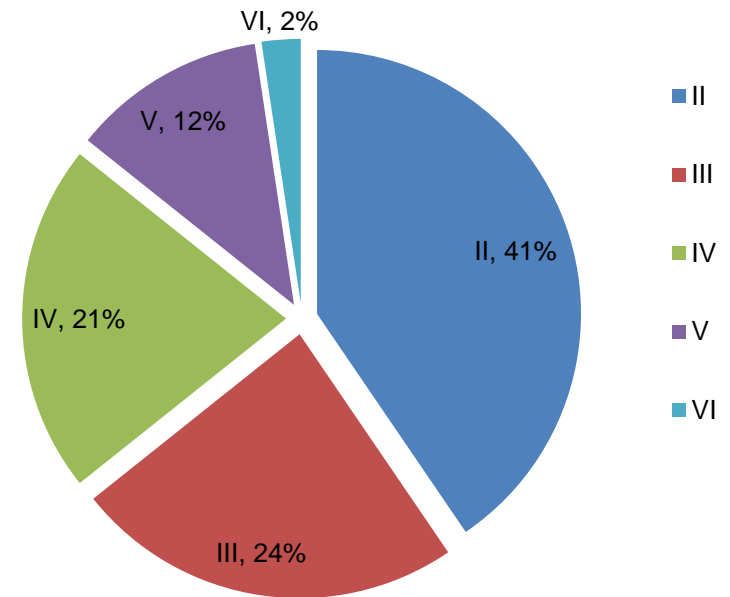
Analysis of whole genome - rep-PCR & analysis of *recA* gene sequence

# Characterization of isolated strains analysis of whole genome - REP profiling

Comparison of rep-PCR profiles  
of Pwa strains isolated in 2013



Distribution of Pwa REP profiles in  
year 2013



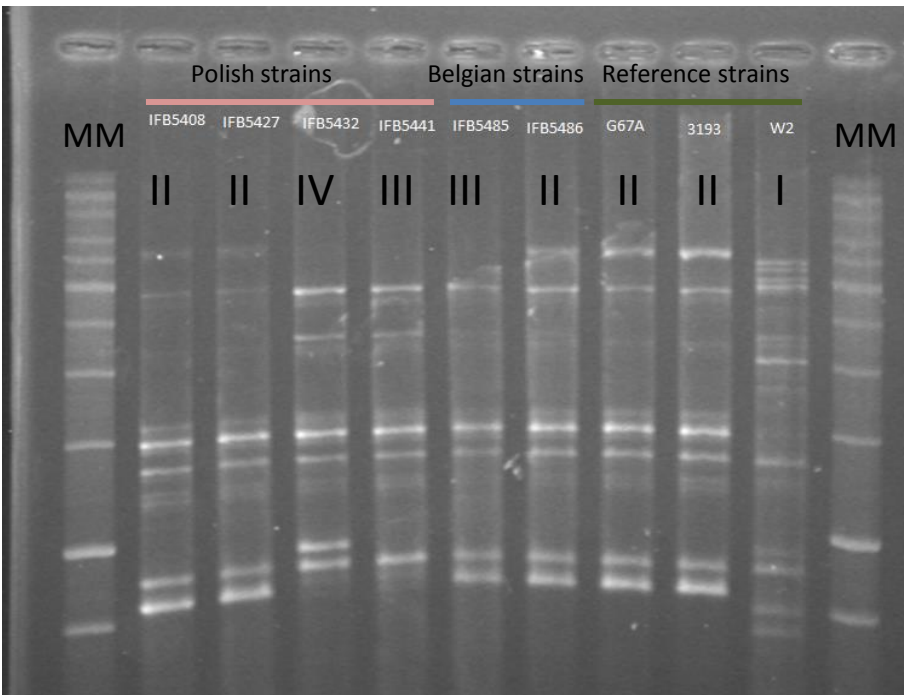
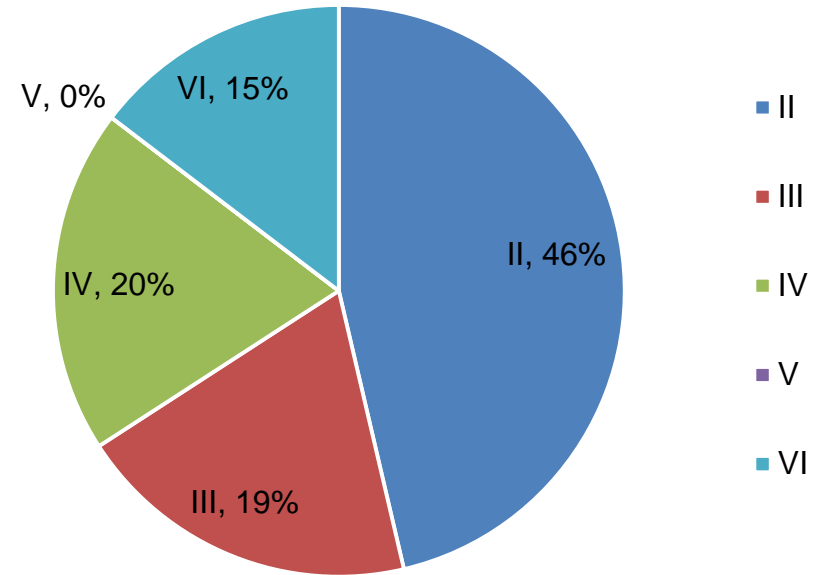
**CFBP 3304 – REP profile I**  
**SCC 3193 – REP profile II**

Repetitive Sequence-Based PCR,  
REP, BOX and ERIC – primers, (Versalovic et al. 1991)



# Characterization of isolated strains analysis of whole genome - REP profiling

REP profiles in year 2014

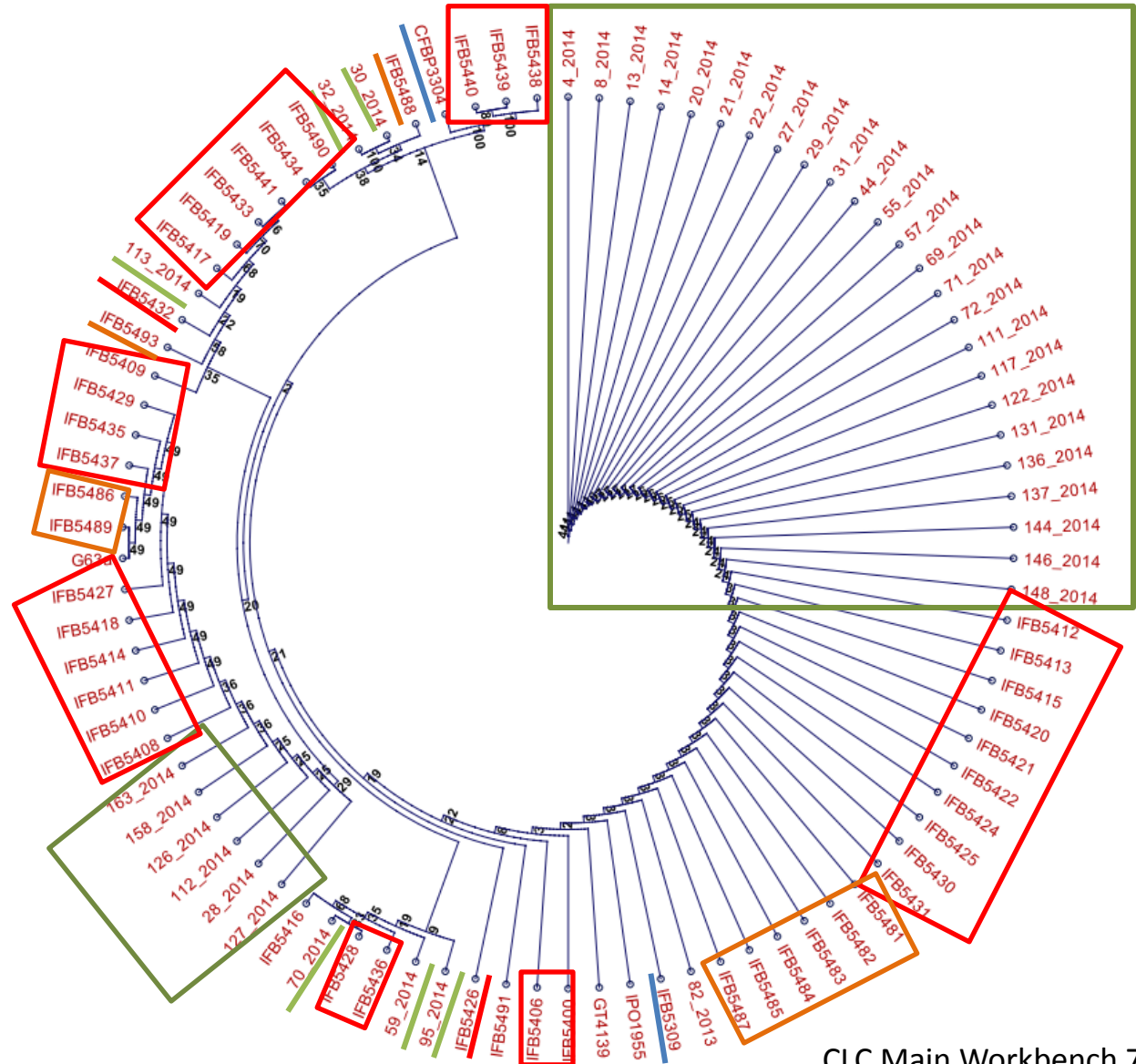


In Polish *P. wasabiae* strains we can distinguish 5 REP profiles ( II – VI), based on REP - PCR

# Characterization of isolated Pwa strains

## – analysis of *recA* sequence analysis

- *recA* sequencing  
680 bp  
Neighbour-Joining  
Jukes-Cantor  
Bootstrap 100 replicons



# Molecular diversity indexes

- Analysis of *P. wasabiae* population from year 2013 vs. population from year 2014
- *recA* based analysis of molecular diversity among two populations

Statistics	2013	2014
Number of substitutions	5	9
Number of transitions	3	4
Number of transversions	2	5
Number of indels	0	0

# Molecular diversity indexes – analysis of strains isolated from the same plant

2013		
Cases of co-isolation		3
Plant	Strains	Pairwise differences
1	IFB5427 IFB5429	0.000000
2	IFB5430 IFB5431	0.000000
3	IFB5432 IFB5433 IFB5434	Number of observed transitions: 1 Number of observed transversions: 1
4	IFB5435 IFB5436	0.000000

2014		
Cases of co-isolation		13
Plant	Strains	Pairwise differences
1	27_2014 28_2014	Number of observed transversions: 1
2	29_2014 30_2014	Number of observed transitions: 1 Number of observed transversions: 3
3	31_2014 32_2014	Number of observed transitions: 1 Number of observed transversions: 3
4	8_2014 14_2014	0.000000
5	13_2014 14_2014	0.000000
6	20_2014 22_2014	0.000000
7	55_2014 59_2014	0.000000
8	69_2014 70_2014 71_2014	0.000000
9	117_2014 136_2014	0.000000
10	131_2014 137_2014	0.000000
11	111_2014 126_2014	0.000000
12	158_2014 163_2014	0.000000

# **PHENOTYPIC CHARACTERIZATION**

# Virulence factors

Motility

Biofilm

Toxins

Siderophores

Pectinases

Cellulases

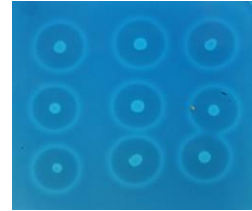
Proteases

LPS

Successive invasion of a  
plant

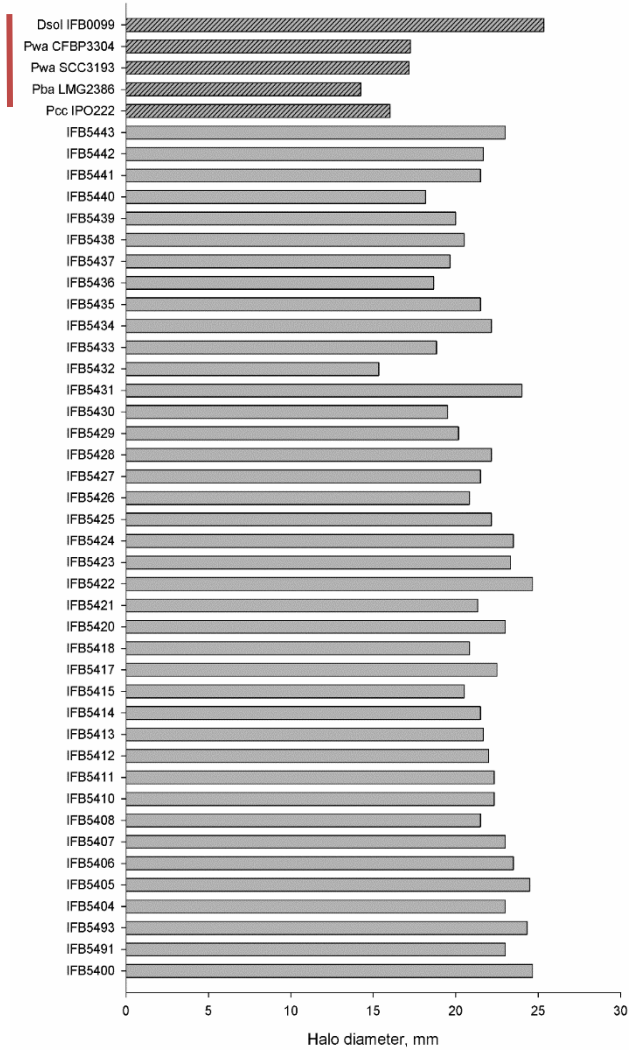


# Characterization of isolated strains – pectinase activity

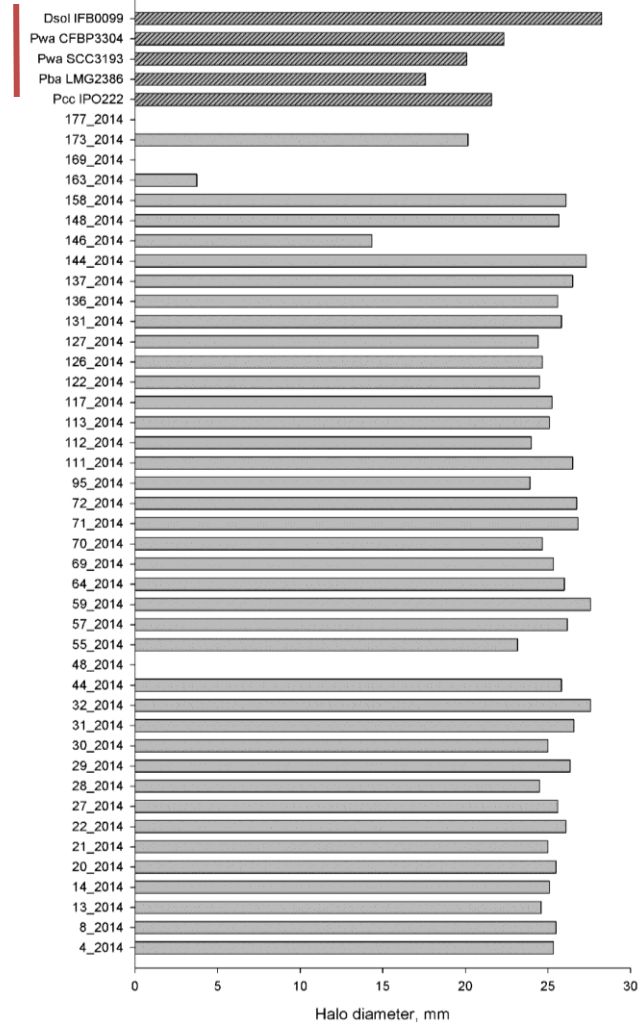


M63 + PGA

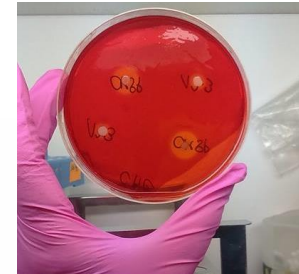
Year 2013



Year 2014

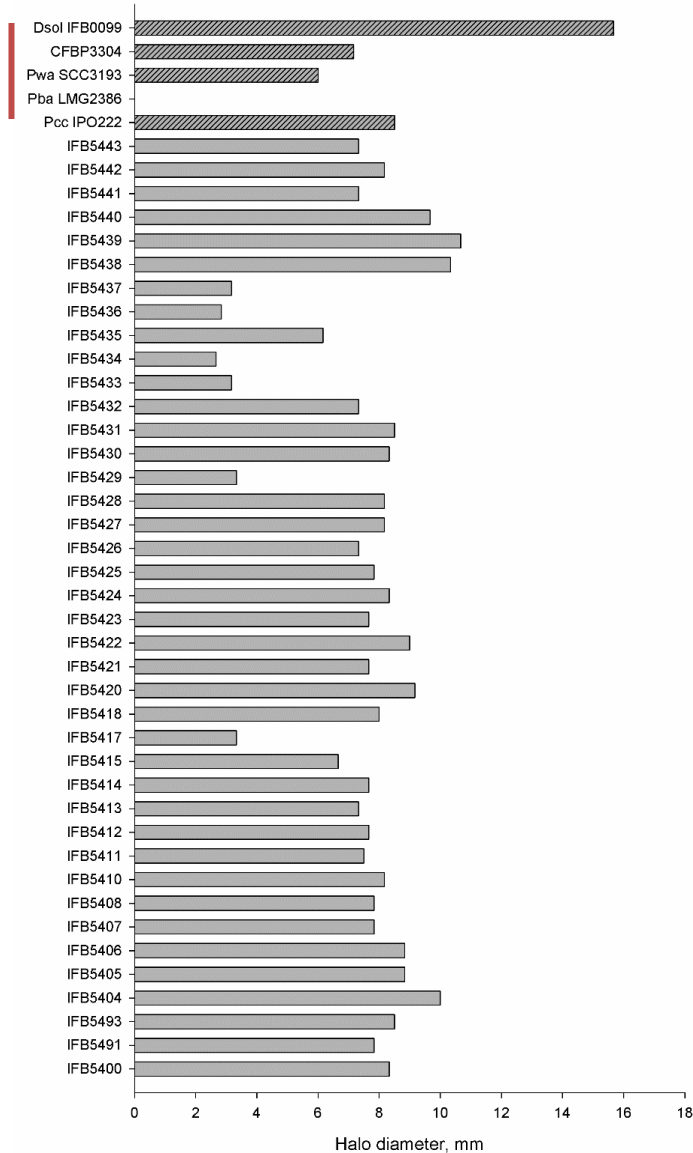


# Characterization of isolated strains – cellulase activity

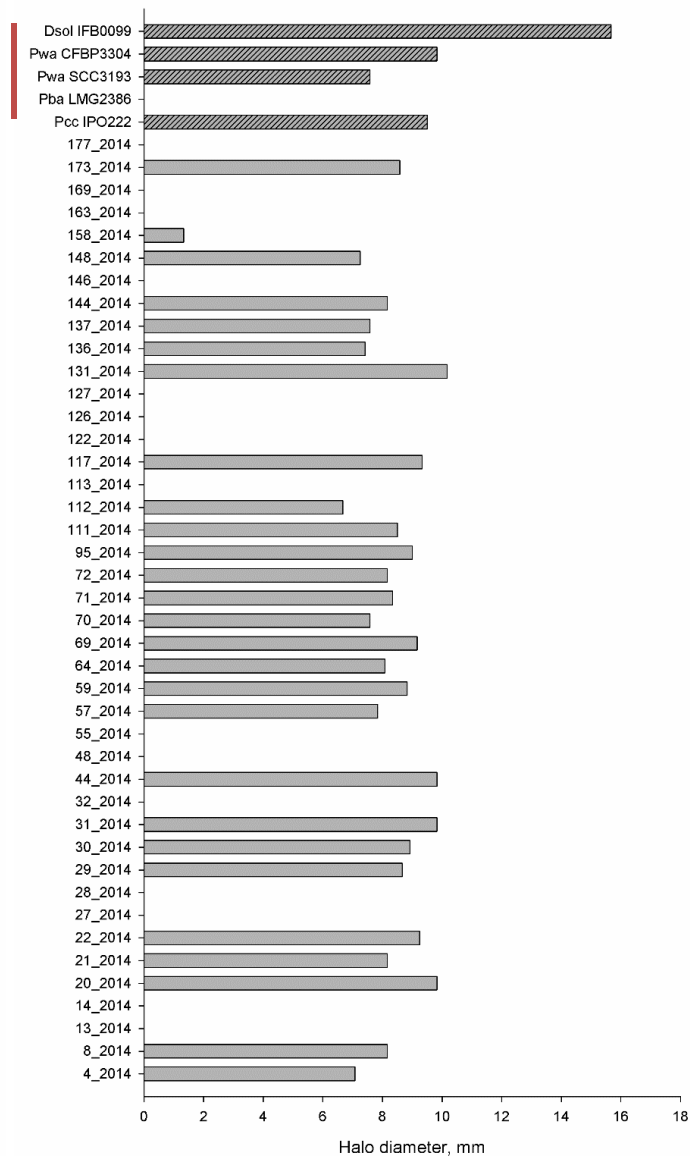


M63 + CMC

## Year 2013



## Year 2014



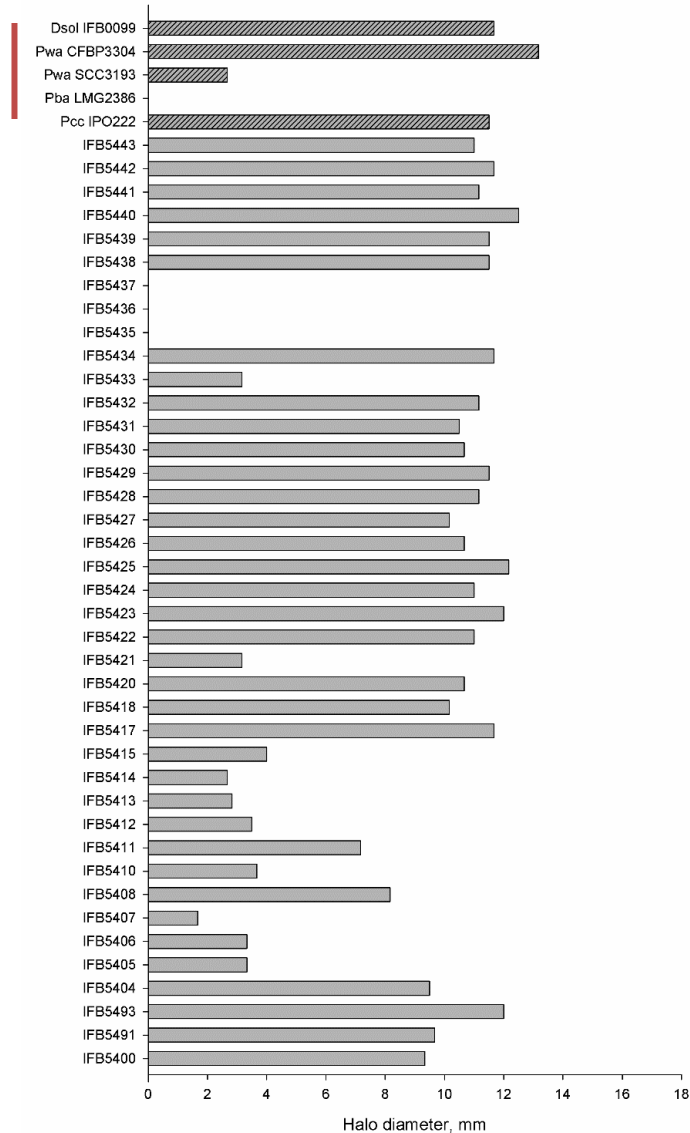


# Characterization of isolated strains – protease activity

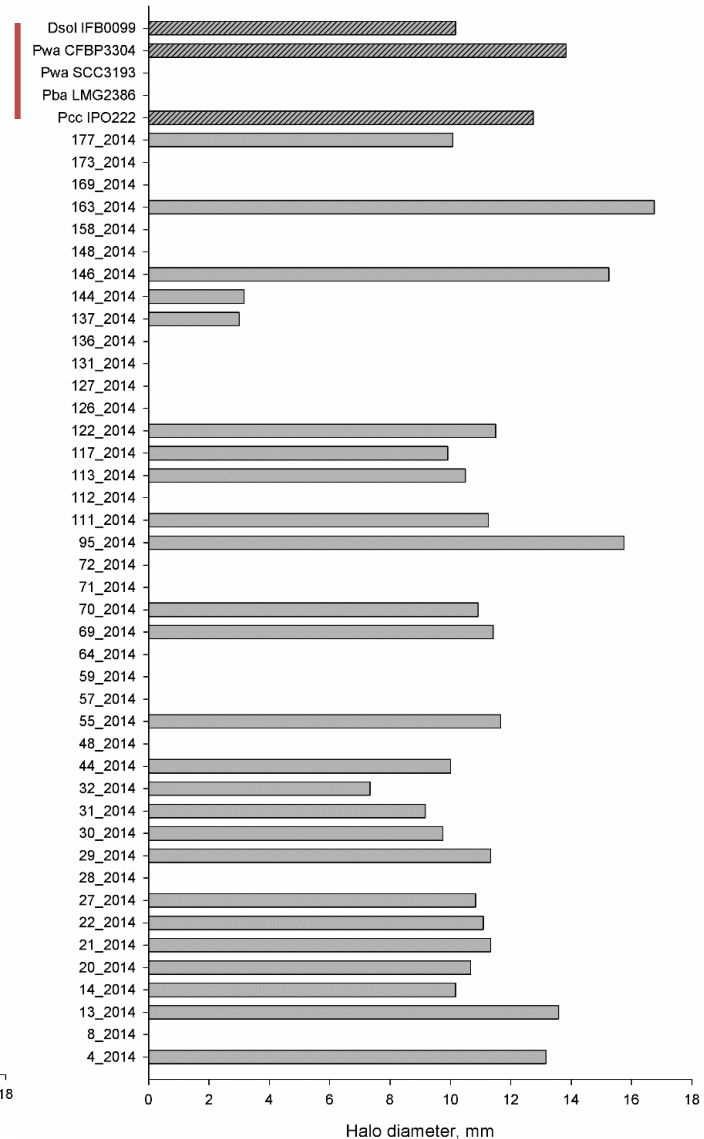


NB + skim milk

Year 2013

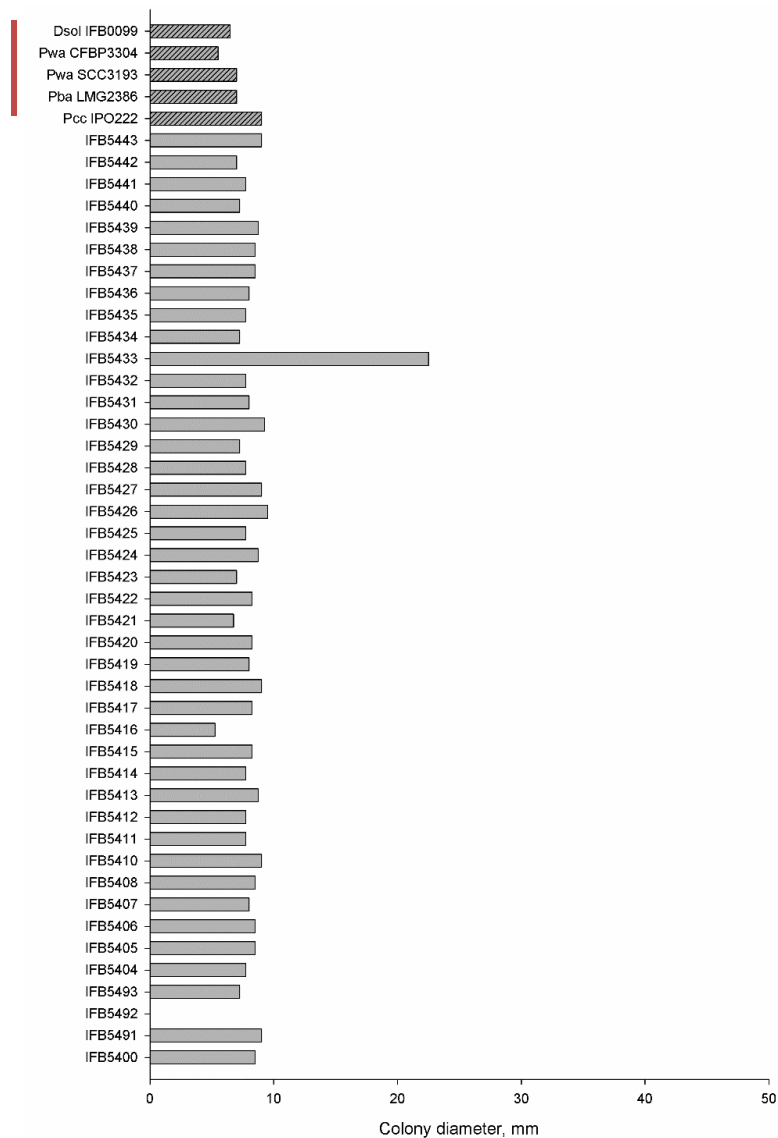


Year 2014

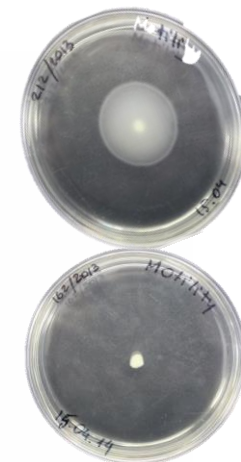
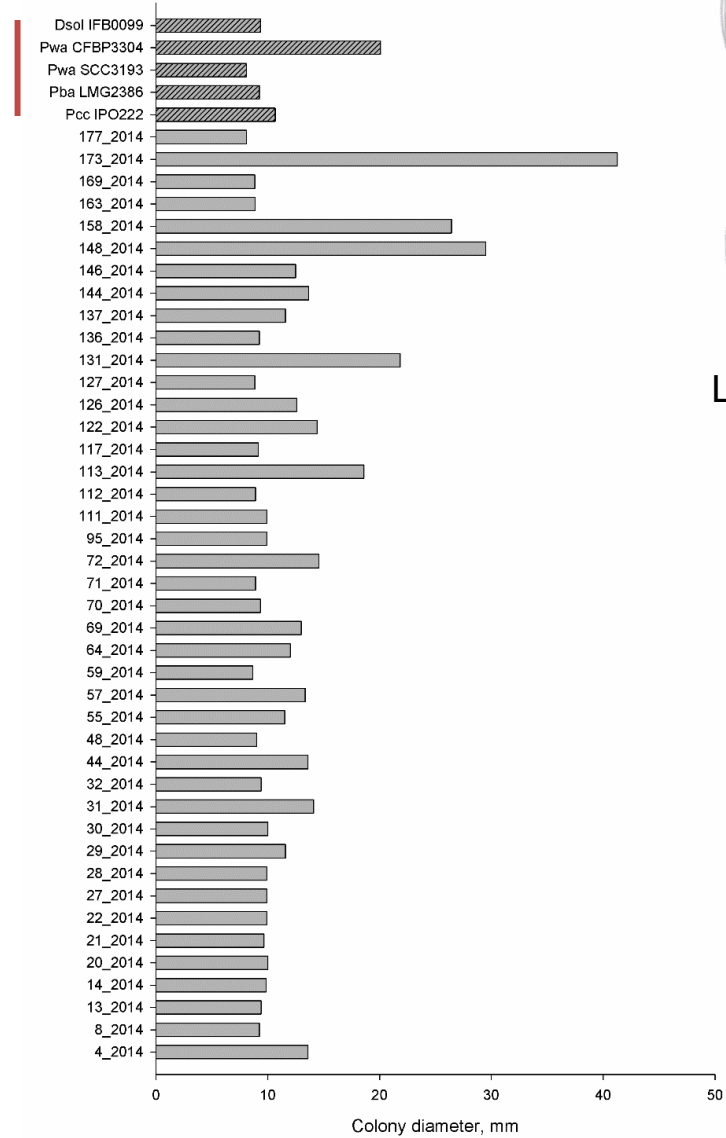


# Characterization of isolated strains – swimming on 0.6% agar

Year 2013



Year 2014



LA + 0.6% agar

# Comparison of phenotypic traits between populations

		2013	2014
Pectinase activity	No activity	0	3
	Low activity	1	2
	High activity	39	37
Cellulase activity	No activity	1	15
	Low activity	6	1
	High activity	33	26
Protease activity	No activity	3	17
	Low activity	10	2
	High activity	27	23
Swarming on 0,6% agar	No activity	1	0
	Low activity	20	21
	High activity	19	21



# Summary

- Bacteria from the genus *Pwa* constitute about 30% of Polish population of pectinolytic bacteria
- Polish population of *Pwa* can be characterized by 5 REP profiles; profile II is the most common one
- Three polymorphic sites in *recA* sequence amplified from genomes of *Pwa* strains isolated in Poland were identified: at 111 bp, 213 bp and 616 bp
- Transversions among *recA* sequence in *P. wasabiae* are more common than transitions
- *Pwa* strains isolated in year 2014 are more variable phenotypically than *Pwa* strains isolated in 2013
- We can speculate that the differences between tested populations are connected with higher transfer of seed potatoes among market

# Thank you for your kind attention



Foundings:  
NSC, Poland, Harmonia 6

POTPAT Pol-Nor/202448/28/2013

