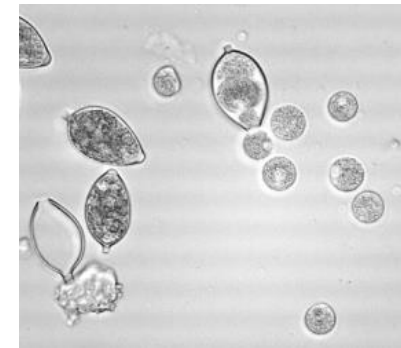




The potato microbiome and its potential impact on late blight resistance



Mout De Vrieze

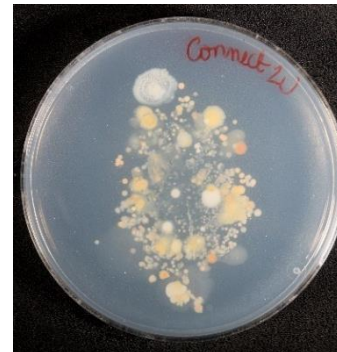
Agroscope, Institute for Plant Production Sciences, Wädenswil & Nyon

08.08.2016

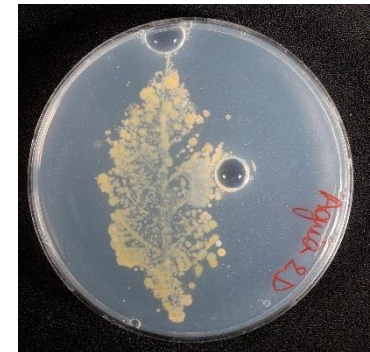


Exploring the microbiome

- The microbiome : not only in the soil and rhizosphere
 - The phyllosphere is a harsh environment but still 10^7 microbes per cm^2 of leaf
 - Impact on their host
- Diversity of microorganisms...
 - ✓ Selected by the plant
 - ✓ Potentially modified upon infection with a pathogen
 - ✓ Potentially beneficial and/or protective...



Healthy resistant cultivar



Symptomatic moderately susceptible cultivar

...against late blight?

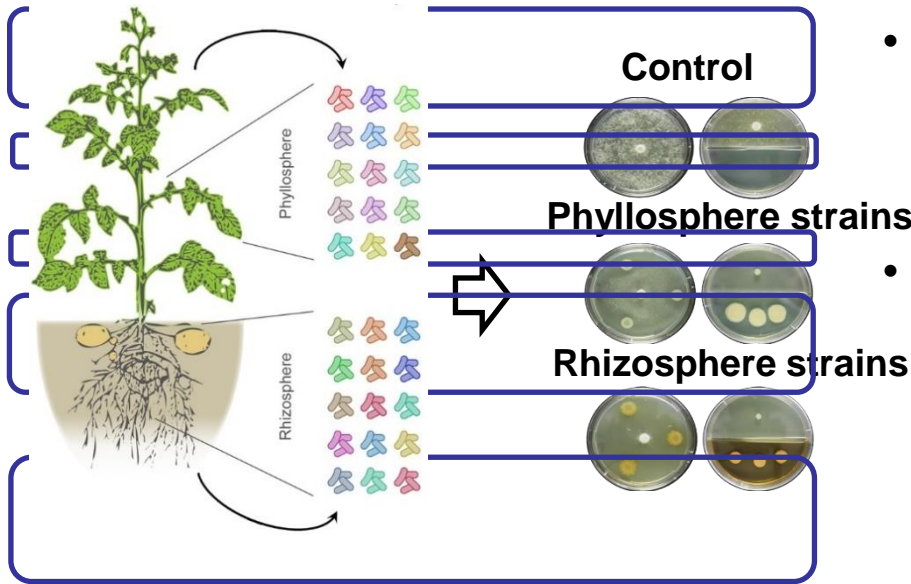


Can we find potential biocontrol strains in the potato's microbiome?





Isolation of microbiome inhabitants



- Overall most active strains belong to the genera *Pseudomonas*, *Bacillus*, *Arthrobacter* and *Microbacterium*

- Pseudomonas* accounts for the most active strains

Selected 15 *Pseudomonas* strains with varying anti-*Phytophthora* activity

Strain	Affiliation	<i>P. inf</i>	<i>H. sol</i>	<i>R. sol</i>	<i>F. oxy</i>	<i>D. dian</i>
R47	<i>P. chlororaphis</i>	8	11	69	81	35
R32	<i>P. vancouverensis</i>	40	41	51	88	41
R84	<i>P. marginalis</i>	22	36	8	77	38
R52	<i>P. marginalis</i>	15	12	91	82	73
S40	<i>P. frederiksbergensis</i>	57	53	79	79	51
R73	<i>Bacillus sp.</i>	7	27	94	84	88
R54	<i>Bacillus sp.</i>	13	18	98	83	87
S50	<i>P. moraviensis</i>	10	66	76	91	58
R10	<i>P. putrefaciens</i>	40	65	104	91	81
R01	<i>P. moraviensis</i>	30	66	95	98	33
R76	<i>P. putrefaciens</i>	25	38	91	93	81
S35	<i>P. marginalis</i>	27	67	81	80	73
S06	<i>P. frederiksbergensis</i>	101	21	82	99	66
R24	<i>P. frederiksbergensis</i>	92	22	95	85	75
S22	<i>P. syringae</i>	85	22	81	93	89
S19	<i>P. frederiksbergensis</i>	40	21	89	91	73
R95	<i>P. lili</i>	72	21	93	99	97
R02	<i>P. veronii</i>	56	60	88	91	92
S46	<i>Curtobacterium sp.</i>	35	78	95	84	97

- Are there potential biocontrol agents among them?

S04	<i>P. frederiksbergensis</i>	97	22	97	98	91
R61	<i>Arthrobacter sp.</i>	8	113	115	99	92
S25	<i>Curtobacterium sp.</i>	77	71	90	98	93
R60	<i>Arthrobacter sp.</i>	15	132	112	87	93
R75	<i>Flavobacterium sp.</i>	82	95	98	86	88
R42	<i>Microbacterium sp.</i>	14	153	107	92	89
R96	<i>Flavobacterium sp.</i>	111	96	99	93	80



Lukas Hunziker



Denise Bönisch



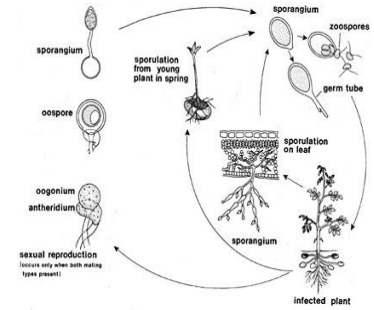
Exploring the bacteria's potential

Mode of action

- What stage of the *Phytophthora* lifecycle do the bacteria affect?
- How are these life stages affected?

Colonisation ability

- Is the anti-*Phytophthora* activity maintained on leaf tissue?
- Are the strains capable of establishing themselves on potato plants?

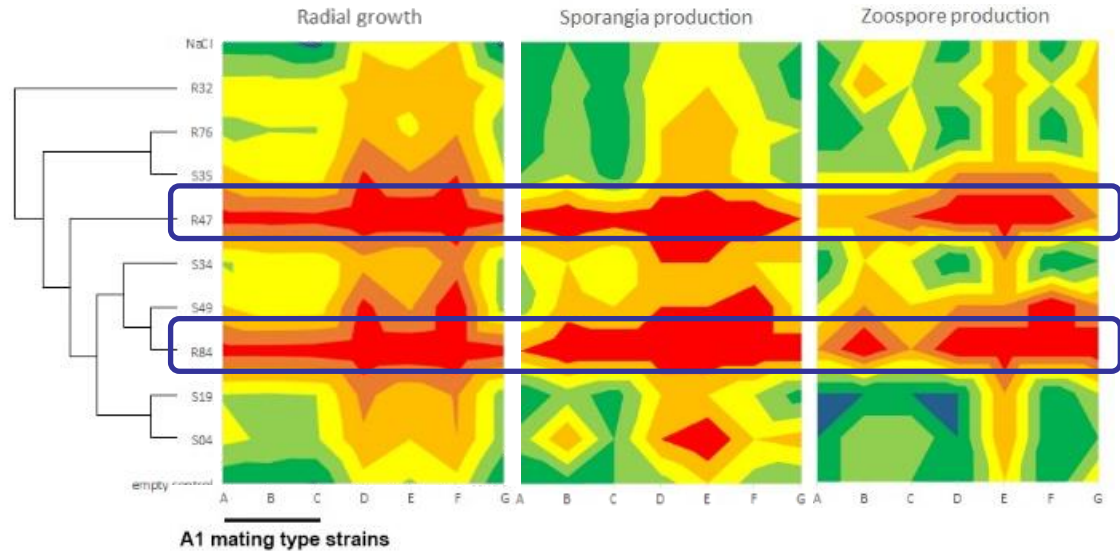
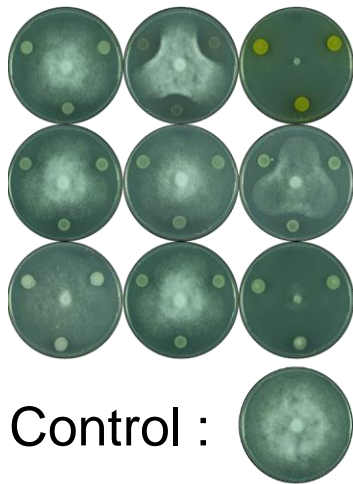
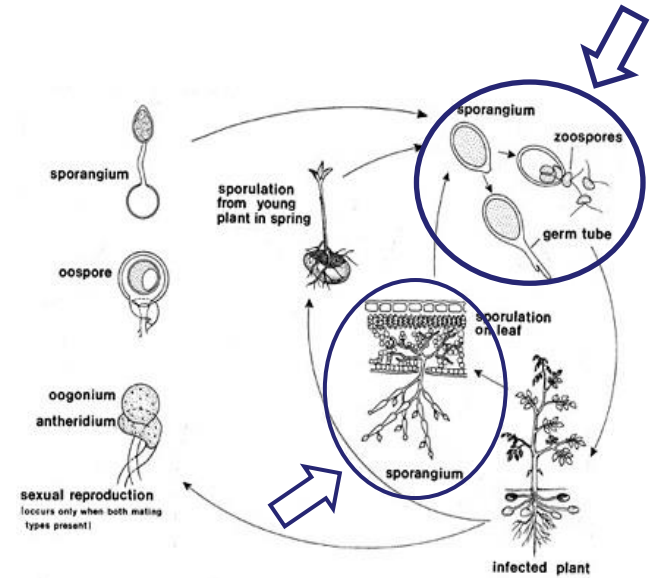


→ Bio assays



Mode of action

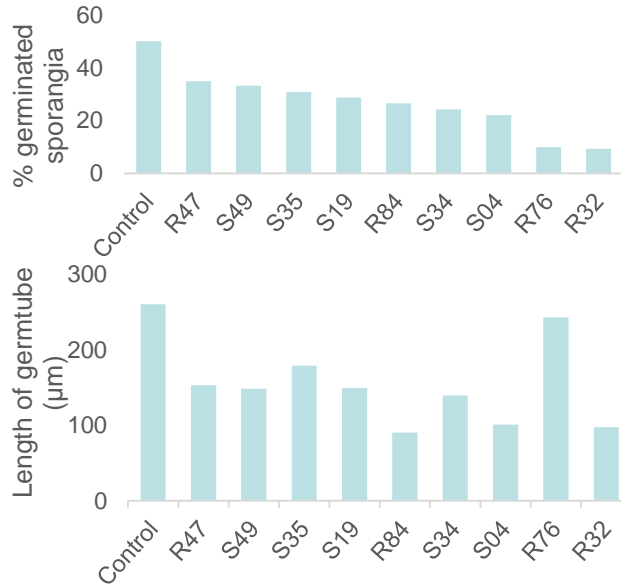
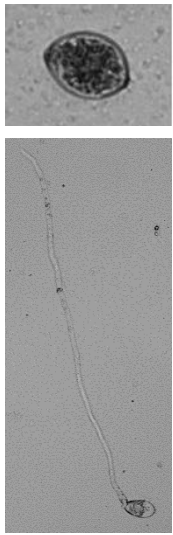
- Effects of the bacteria on different stages of the *P. infestans* life cycle :
- ✓ Mycelial growth
- ✓ Sporangia production
- ✓ Zoospore production





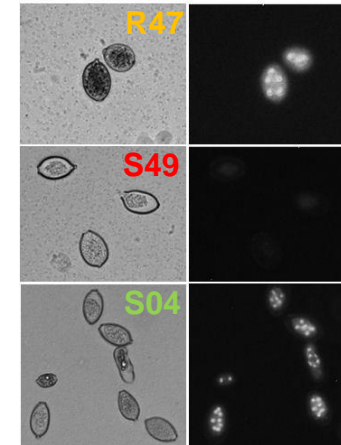
Mode of action

✓ Sporangia germination



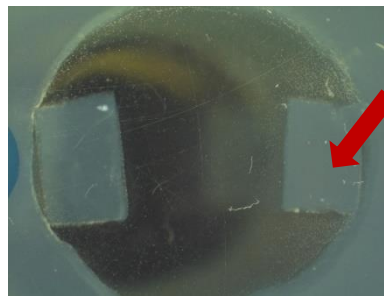
✓ Sporangia viability

→ Propidium iodide staining

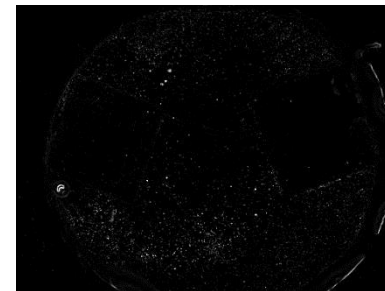


	Alive	Dead
R47	52 %	48 %
S49	98 %	2 %
S04	8 %	92 %

✓ Zoospore motility and chemotaxis



Aspartic acid

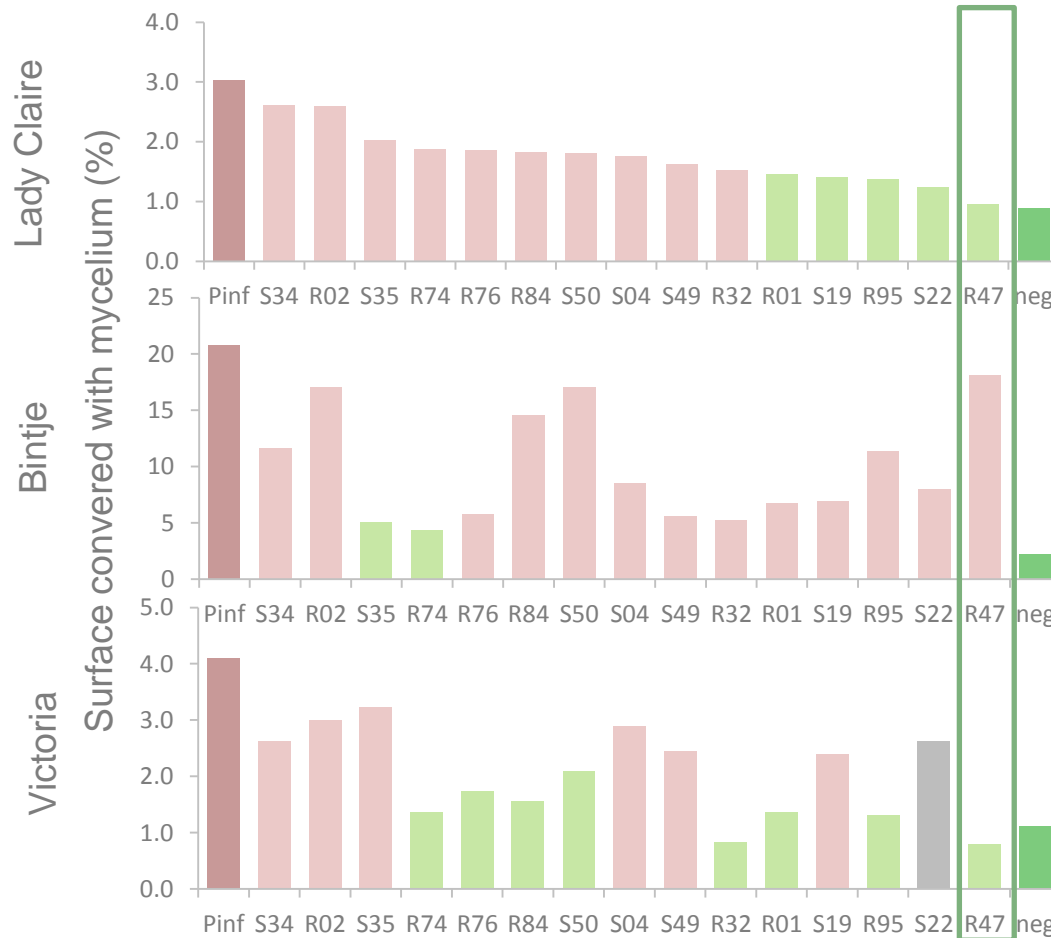
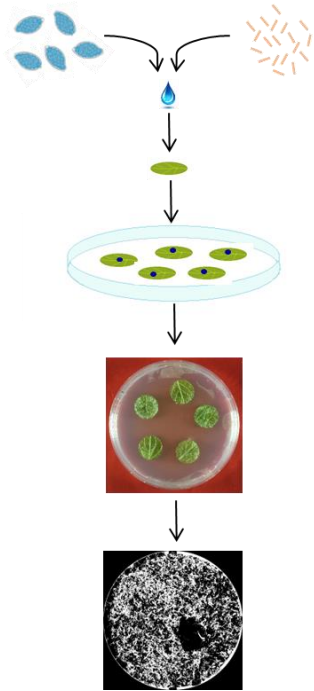


Control

R47 supernatant

Is the anti-*Phytophthora* activity maintained on leaf tissue?

- *In vivo* test on leaf discs : coinoculation on leaf tissue





Can the bacteria colonise potato plants?



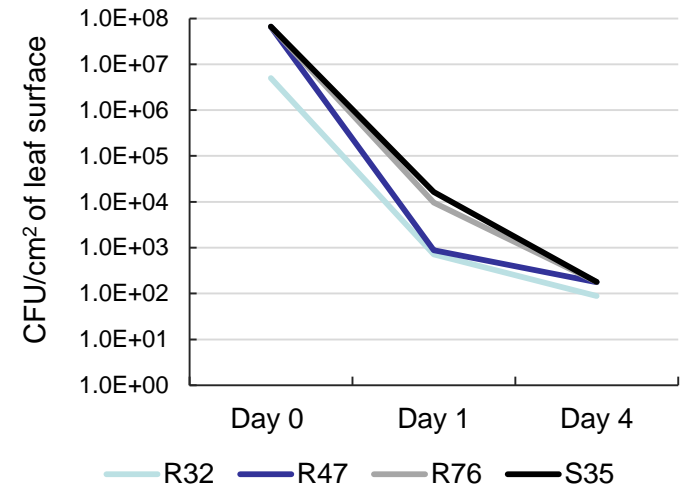
- Tuber inoculation
- ✓ Bacterial suspensions (OD=1)
- ✓ Stem cuts

	Not sterilised	Surface sterilised
R32	••	•••
R47	•••	••
R76	••	••
R84	•	-
S04	•	••
S19	••	••
S34	••	•••
S35	••	•••
S49	••	•••

- retrieved after 3 weeks
- retrieved after 4 weeks
- retrieved after 5 weeks

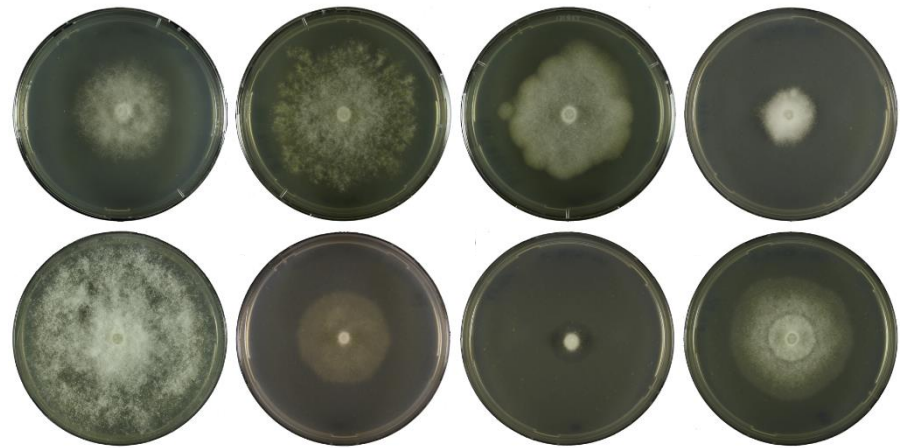


- Leaf application
- ✓ Bacterial suspensions (OD=1)
- ✓ Leaf discs
- ✓ CFU count





*Do different *P. infestans* strains differing in virulence show varying susceptibility to biocontrol bacteria?*



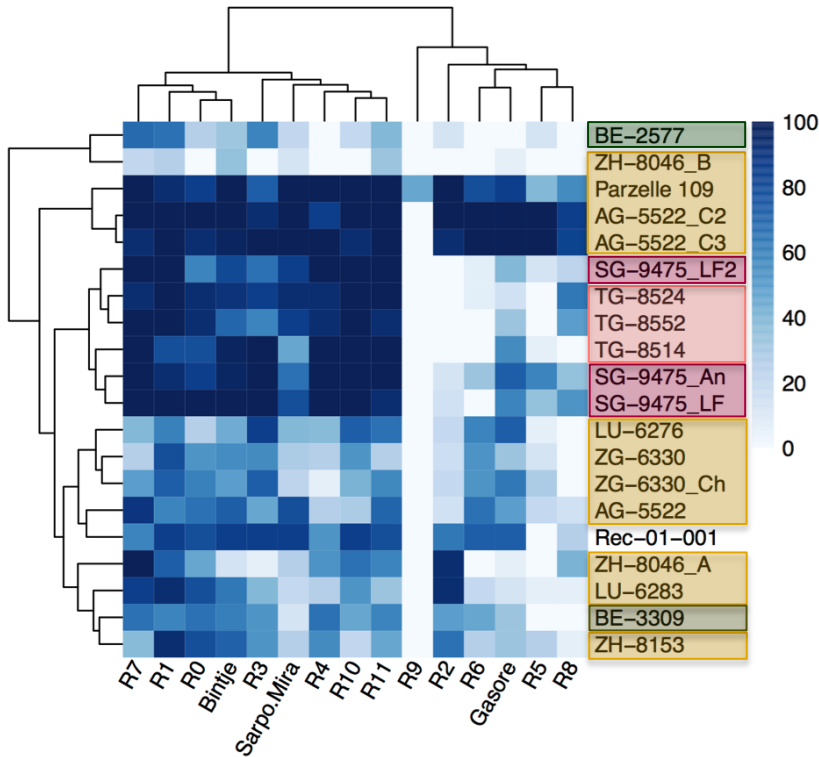


Monitoring of *P. infestans* in Switzerland – Characterisation of virulence



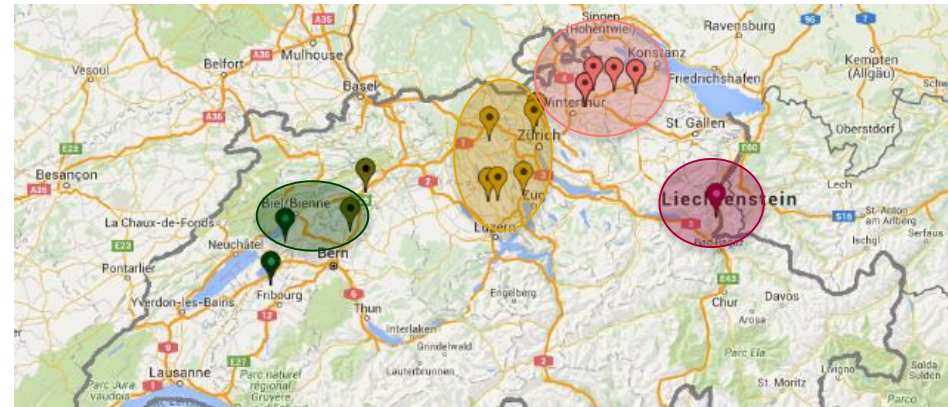
Ramona Gloor

→ Leaf disc assay

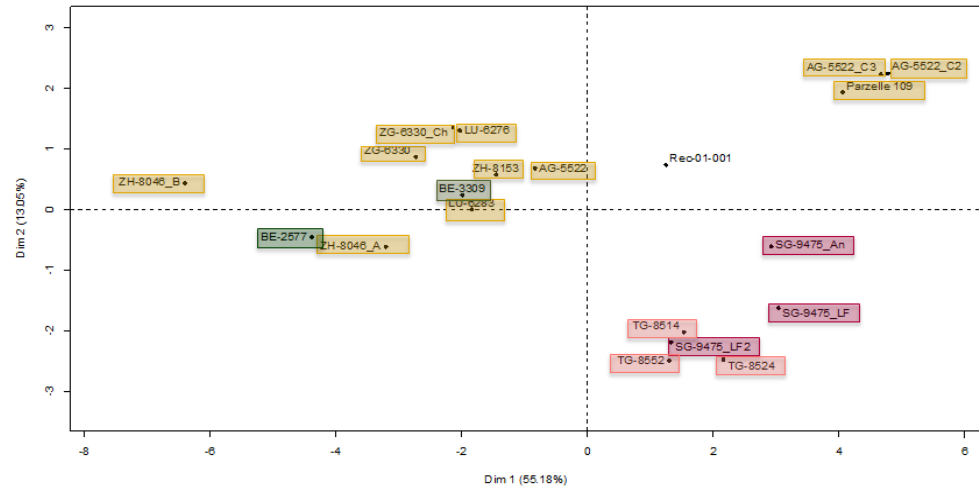


□ 0 leaf discs infected

■ All 15 leaf discs infected



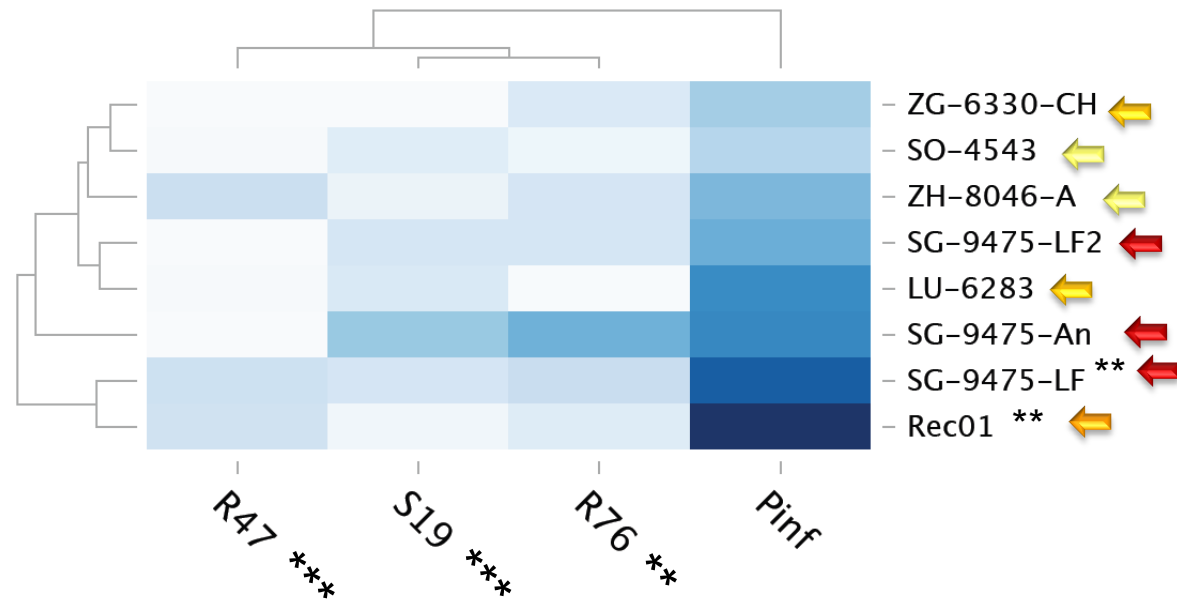
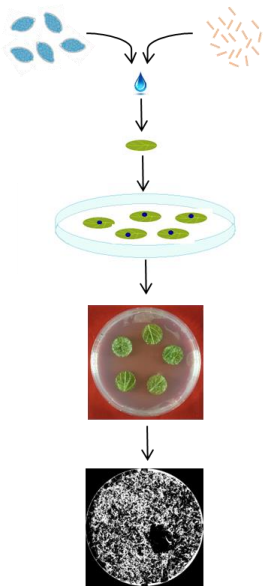
Individuals factor map (PCA)





Susceptibility of *P. infestans* strains varying in virulence towards our antagonists

- Leaf disc assay with subset of *P. infestans* strains



- All *P. infestans* strains are inhibited
- The response varies from bacterial strain to bacterial strain regardless of the apparent virulence of the *P. infestans* strains



Outlook - Mechanisms ?

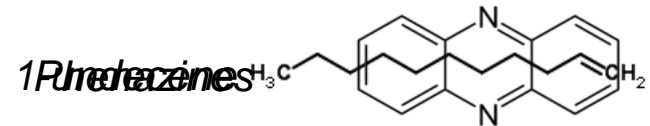
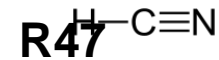


Arviden R. Ballynar

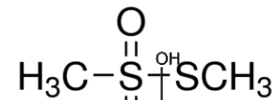


- ✓ CLSA and GC-MS to identify volatile organic compounds (VOCs)
- Diffusible compounds
→ TLC
- Genome mining (9 strains)
→ Looking for genes
- Comparative genomics
→ comparison of genomes of strains showing varying anti-*Phytophthora* activity

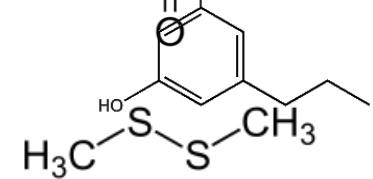
HCN



MMTS

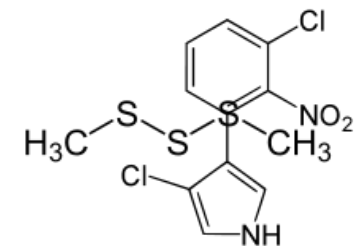


HPR



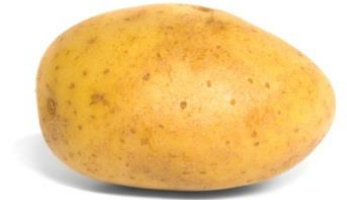
DMDS

DMTS





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Thank you for your attention

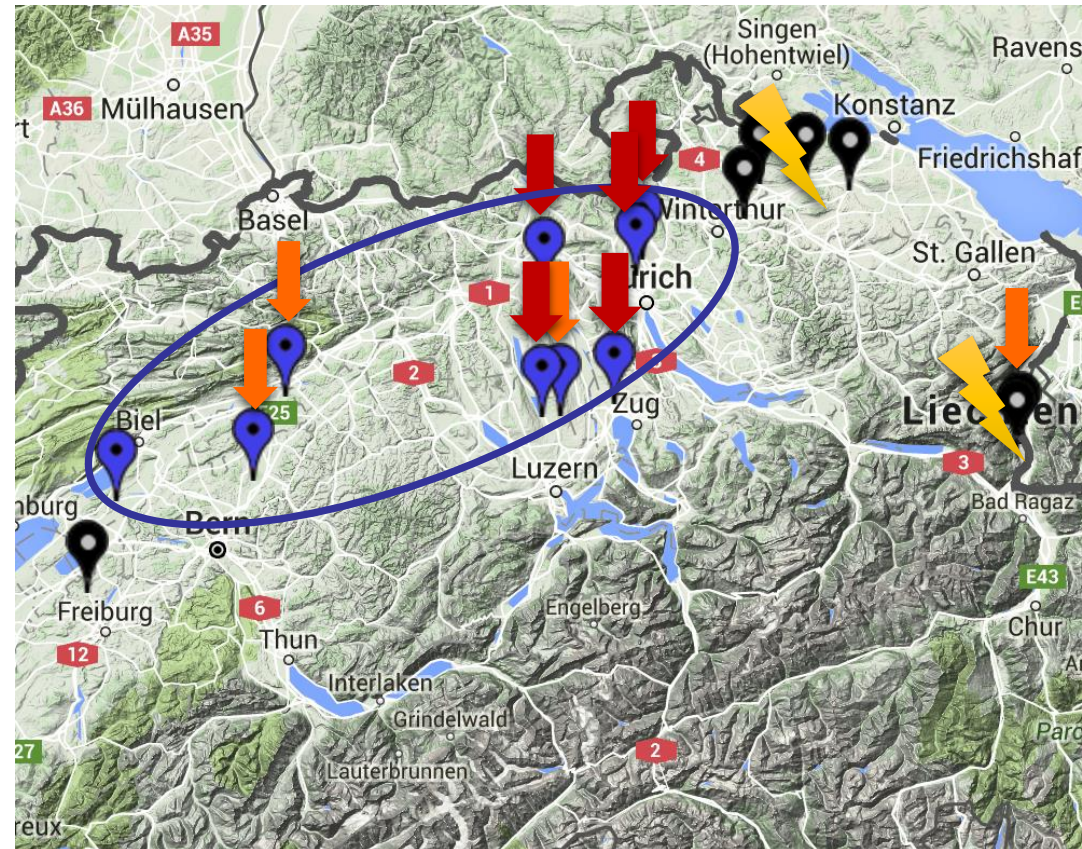



Agroscope good food, healthy environment

“Monitoring of *Phytophthora infestans* population diversity in Switzerland”

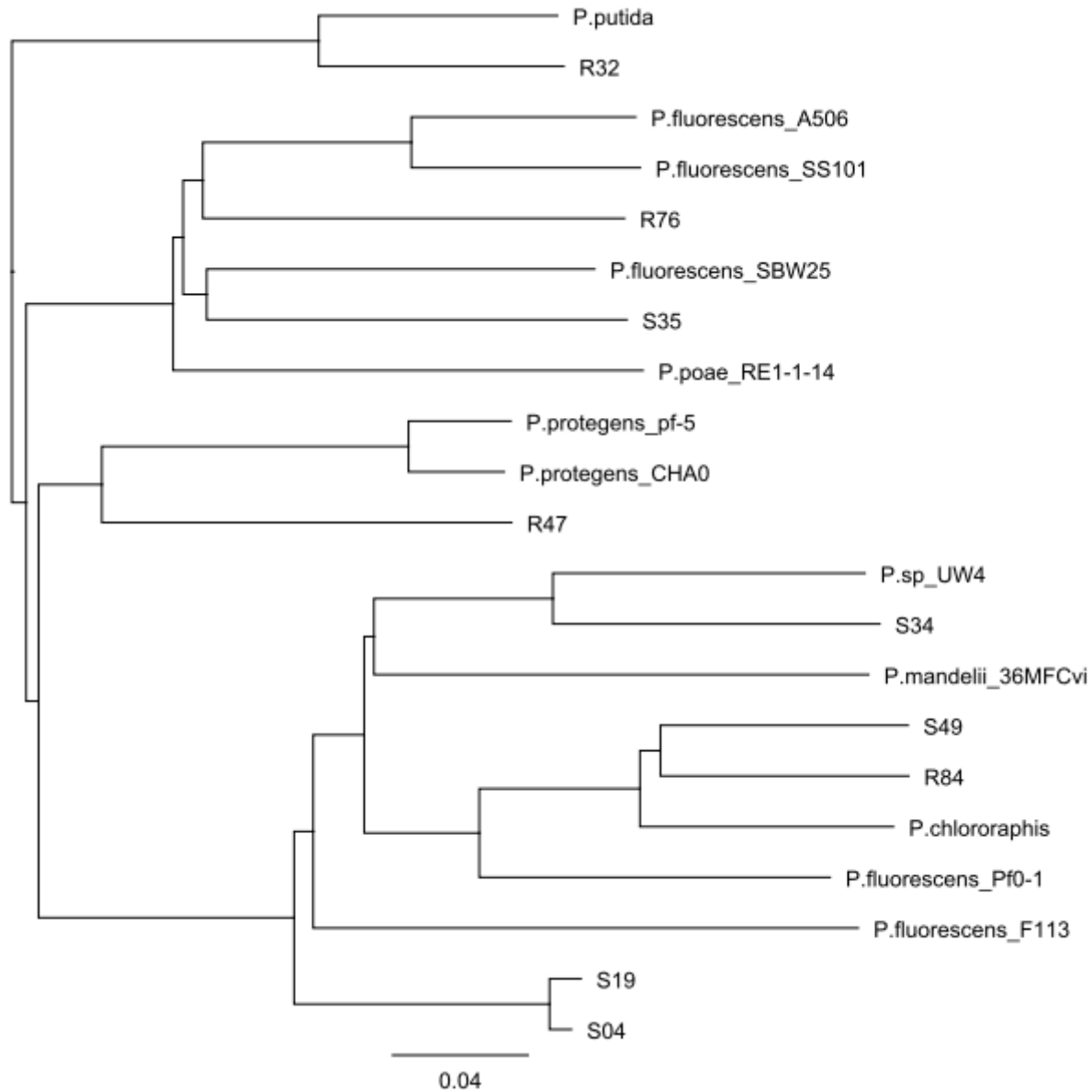
In 2015 :

- ✓ 22 isolates were successfully retrieved
- ✓ 3 A1, 18 A2
- ✓ Majority of EU_13_A2
- ✓ Increased resistance against Mefenoxam in the EU_13_A2 isolates



 EU_13_A2

 miscellaneous





Antagonistic leaf disc assay: method



Anouk
Guyer



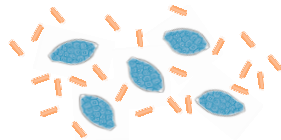
Aurélien
Bailly



Mout
De
Vrieze

Leaf discs

Sporangia-bacterial
suspension



Greenhouse plants

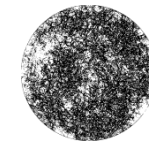
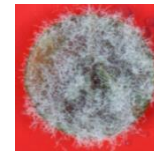


Inoculation of the
leaf disc (Ø 2 cm)

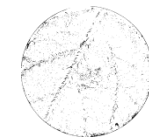
7 days



Evaluation of % the surface
covered with mycelium...



49.4 %



2.8 %

...with automated picture
analysis