

Whole genome sequencing elucidates the genomic background of fungicide resistant and -susceptible *Alternaria solani* strains

#ECFG15



Uhrenturm der TVM



#### Alternaria solani (causes early blight) is of increasining relevance in Germany



## ТШ

#### Development of resistance against strobilurines (Qol´s) and carboxamides (SDHI´s)





#### Incedence of SDHI resistant strains is increasing in Germany





#### SDH mutants in Germany in 2014



- Locations w/ mutants
- Locations w/ only wildtype



#### SDHI resistance caused by pointmutations in Sdh complex

- Affect the mitochondria
  - of A. solani conidia
- -> inhibit electron transport in mitochondrial respiration at complex II (Sdh)
- Binding site is formed by subunits B-C-D



Marcin Sarewicz, Artur Osyczka 2015 Physiol. Rev

Known mutations in A. solani: B: H278R/Y C: H134R D: D123E, H133R

Are there phenotypic differences between the mutants?





Do SDH mutations arise once and then spread?

Resequencing study:

What? Two regions Strains with and without mutations

How? SNP calling to reference genome (NL03003)

Then what? Looking for genetic clustering >What is the genetic variation in SDHI mutants?



#### 20 isolates, 16 from Germany





Half from Bavaria, Half from Lower Saxony



"Dutch" isolates kindly donated by Vivianne Vleeshouwers & Jaap Wolters, Wageningen University



Half with SDH mutation, Half without







Tamara Susanto

#### We recover the SDH mutations

		) . 8	310	820	830	840
NL03003_As_Chr7g04610.1_SDH_B/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CÁCACCÁTTC	TCAACTOCTC
As_615.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_628.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_676-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_687-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_691-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_692-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_711-2.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	TACACCATIC	TCAACTGCTC
As_720-3.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	TACACCATIC	TCAACTGCTC
As_732-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_736-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_737-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	TACACCATIC	TCAACTGCTC
As_739-3.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_746-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_749-3.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	CACACCATIC	TCAACTGCTC
As_754-2.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	TACACCATIC	TCAACTGCTC
As_774-1.SDHB/1-921	CAGGATGCTCTC	AACAACAG	CATGAGCTTG	TACCGATGC	TACACCATTC	TCAACTGCTC

	370	380	390	400	410	420	430
WL03003_As_Chr2g12560_SDH_C/1-534	CTCÁAGGCŤ	TTCTACGCTT	тессёттеті	CTTCCACAGO	TTCAACGGCT	TGAGGCATCT	этостве
As_615.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCC <mark>A</mark> CAGC	TTCAACGGCT	TGAGGCATCT	зтостве
As_628.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCC <mark>G</mark> CAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_676-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_687-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCGCAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_691-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCC <mark>G</mark> CAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_692-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCC <mark>G</mark> CAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_711-2.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	STOCTES
As_720-3.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтостор
As_732-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCC <mark>G</mark> CAGC	TTCAACGGCT	TGAGGCATCT	этост <mark>бб</mark>
As_736-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_737-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_739-3.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_746-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_749-3.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтост <mark>бб</mark> і
As_754-2.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтостве
As_774-1.SDHC/1-534	CTCAAGGCT	TTCTACGCTT	тесссттетт	CTTCCACAGC	TTCAACGGCT	TGAGGCATCT	зтостве

#### SDHC



## Our samples form three groups





### Some samples are very similar



Tamara Susanto



Tamara Susanto



#### SDH mutations might seem to originate in different genetic backgrounds



### SDH mutations might seem to originate in different genetic backgrounds



#### Tamara Susanto

#### Establish the number of distinguishable genotypes: 4 (or 5)





Genotypes, partly geographical?





#### Genotypes, partly defined by SDH mutations





Fitness, linked to SDH mutation?





Fitness, linked to "genotype"





Conclusions:

SDH mutants belong to different genotypes So, potato trade did NOT spread the resistance!

H134R mutation arose at least twice (or three times?)

No trade off between mutation & virulence on this level yet "Genotypes" might define fitness of the strains





**Open Questions:** 

Besides SDH, which other genes are affected?

How do they define fitness? Can a agressive SDH mutant arise easily?

These are ~2015 strains, what happens now? Changes over time? One dominant SDH mutant strain? Do we see develpment of new fitness effects?

This is a German / USA sample set. What are the backgrounds elsewhere in Sweden? Belgium? Poland?

H134R mutation in many genetic background, suggest it arose more than once. What are the implications?

Mutations against other fungicides also arose before / after What is their effect on the population genetics?



Next steps

Looking for collaborators!

> Exand sample sets

> Develop high throughput phenotyping

Link genetic diversity to life traits

Dive deeper into these low / high variation regions on the genomes

Follow-up study: are the fitter mutants spreading faster? Sample 2020!





# Acknowledgements

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Poster B2-07

Genetic diversity of Alternaria in natural populations

