

Global and regional diversity in *Alternaria* causing potato early blight

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Introduction & State of the Art

- One of the most important potato foliar diseases worldwide.
- Significant yield and quality losses (20-50%)
- Historically, the disease has been mainly associated with *Alternaria solani*.

Expanding view of the *Alternaria* complex

- A diverse complex of *Alternaria* species despite typical leaf symptoms
- Molecular tools have revealed diversity
- Studies about diversity are needed to know virulence and fungicide effectiveness





Goals of this study

1. Study the diversity of *Alternaria* species focusing in different regions
2. Characterization of *Alternaria* species
 1. Genetically
 2. Morphologically
3. Study response of *Alternaria* species to fungicide strategies





Methodology



From Farm





....To the lab

1 SAMPLE COLLECTION AND ISOLATION



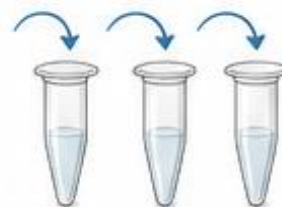
Monitoring of Early blight symptoms in potato fields in A Limia (NW Spain) and Jutland, Denmark (2023 season).



Symptomatic leaves were dried. One spot per leaf was cut and considered as one isolate.



Dried spots were stored until molecular analyses.



Surface disinfection:

- 5% sodium hypochlorite for 1 min
- Rinsed in sterile water for 1 min
- Washed with autoclaved water for 1 min
- Dried on sterile filter paper



Plated on PDA + Novobiocin Sodium Salt (0.1 g/mL) and incubated at 17 ± 1 °C for 5 days.



From NW Spain, 59 isolates grew. Mycelial plug from colony edge transferred to PDB and incubated for 5 days.

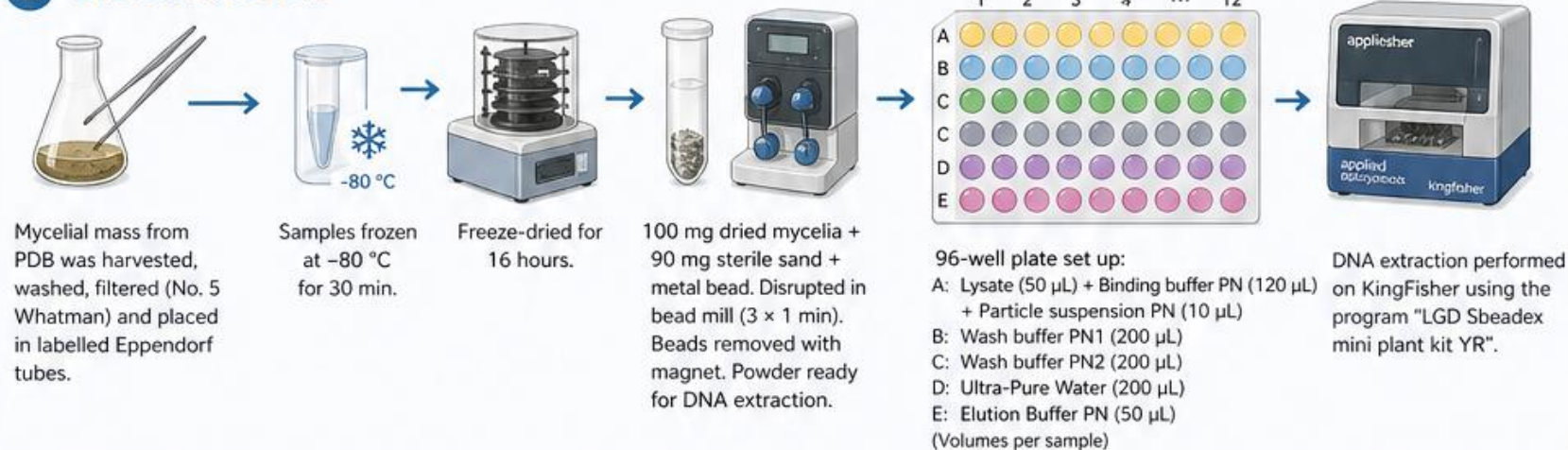


Pure cultures were maintained and stored at -80 °C in the culture collection of the Agroecology Centre, University of Aarhus, Flakkebjerg, Denmark.

Total isolates:
62 (NW Spain)
78 (Denmark)



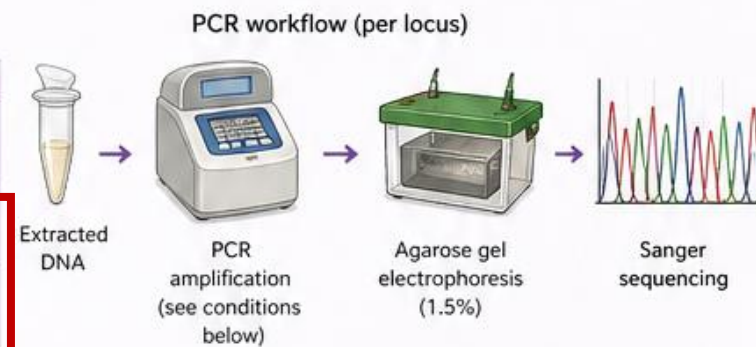
2 DNA EXTRACTION



3 PCR AMPLIFICATION AND SANGER SEQUENCING

Primers used in this study

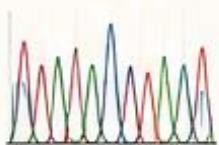
Gene (locus)	Primer	Direction	Sequence (5'-3')	Reference
ITS	ITS1F	Forward	CTTGGTCATTTAGAGGAAGTAA	White et al., 1990
	ITS4	Reverse	TCCTCCGCTTATTGATATGC	
TEF		Forward	CAT CGA GAA GTT CGA GAA GG	Carbone & Kohn, 1999
		Reverse	TAC TTG AAG GAA CCC TTA CC	
RPBII		Forward	GGG GWG AYC AGA AGA AGG C	Sung et al., 2007; Liu et al., 1999
		Reverse	CCC ATR GCT TGT YYR CCC AT	





4 GENOMIC SEQUENCE ANALYSIS

4.1 Sequence analyses



Raw Sanger sequences

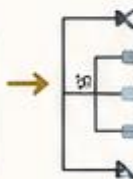
ATGCTAGC
GCTAGCTA
TTGACGTA

Quality check and trimming

ATGCTAGC
ATGCTAGC
ATGTTAGC

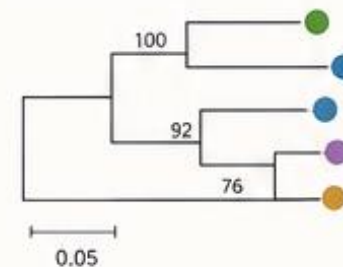
Sequence alignment (ITS, EF, RPBII)

- Species identification
- Haplotype analysis
- Genetic diversity assessment



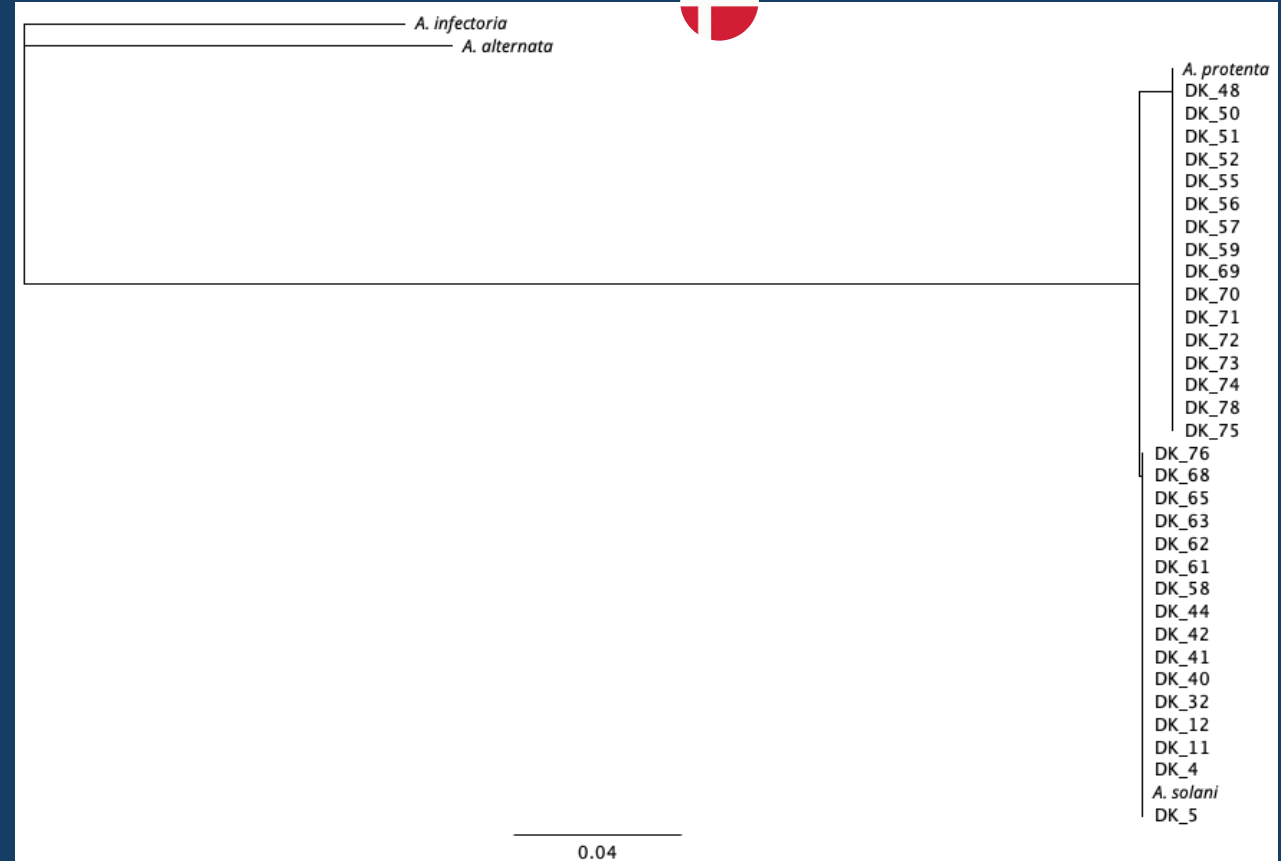
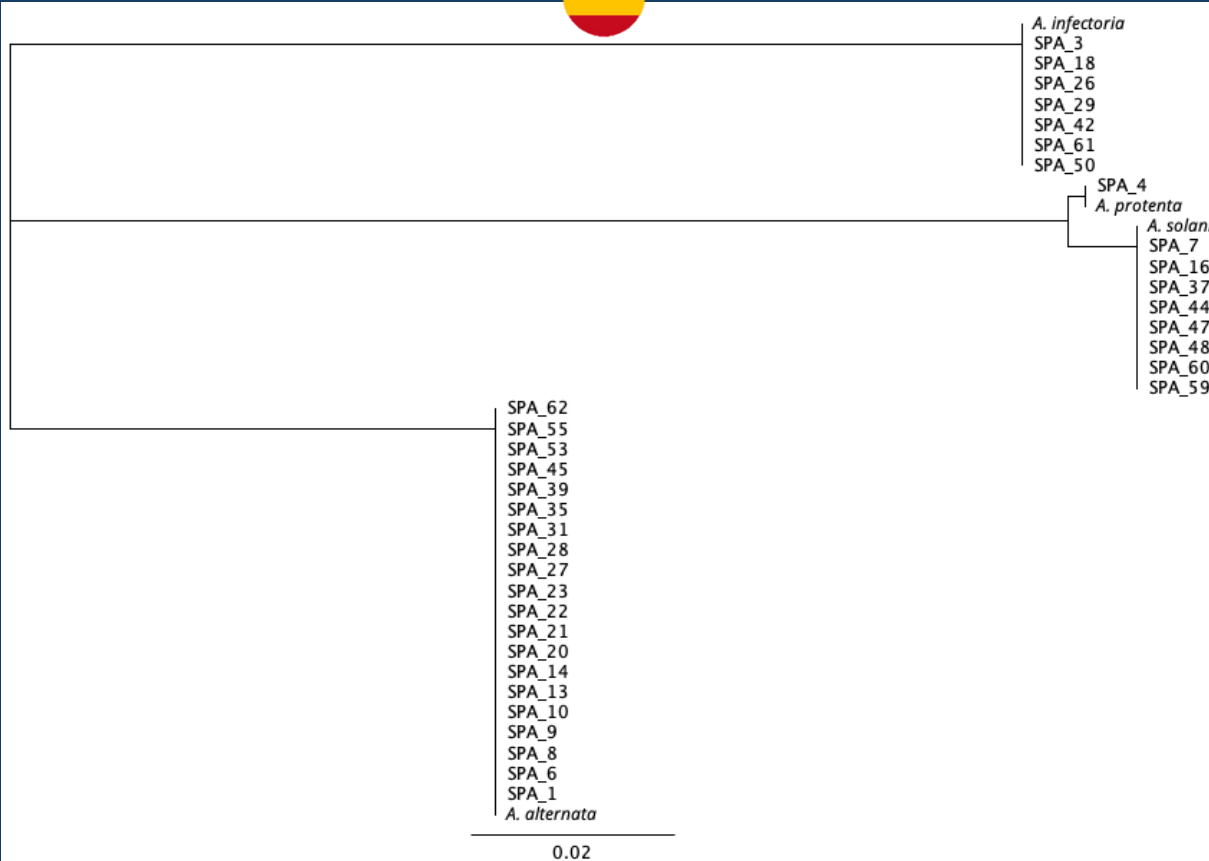
4.2 Phylogenetic analysis

Method: UPGMA – Jukes Cantor
Bootstrapping: 100 replications



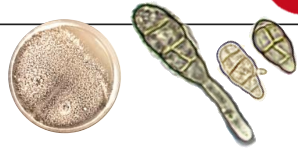


Results: sequencing analyses





Results: sequencing analyses



Infectoria group

A. infectoria
SPA_3
SPA_18
SPA_26
SPA_29
SPA_42
SPA_61
SPA_62

SPA_4
A. protenta
A. solani
SPA_7
SPA_16
SPA_37
SPA_44
SPA_47
SPA_48
SPA_60
SPA_59

Protenta group

Solani group

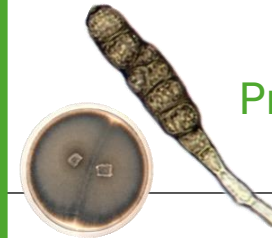


Alternata group

SPA_62
SPA_55
SPA_53
SPA_45
SPA_39
SPA_35
SPA_31
SPA_28
SPA_27
SPA_23
SPA_22
SPA_21
SPA_20
SPA_14
SPA_13
SPA_10
SPA_9
SPA_8
SPA_6
SPA_1
A. alternata

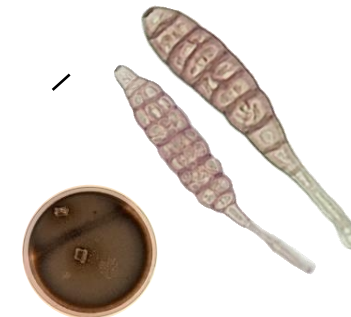
0.02

A. infectoria
A. alternata



Protenta group

A. protenta
DK_48
DK_50
DK_51
DK_52
DK_55
DK_56
DK_57
DK_59
DK_69
DK_70
DK_71
DK_72
DK_73
DK_74
DK_78
DK_75



Solani group

DK_76
DK_68
DK_65
DK_63
DK_62
DK_61
DK_58
DK_44
DK_42
DK_41
DK_40
DK_32
DK_12
DK_11
DK_4
A. solani
DK_5

0.04

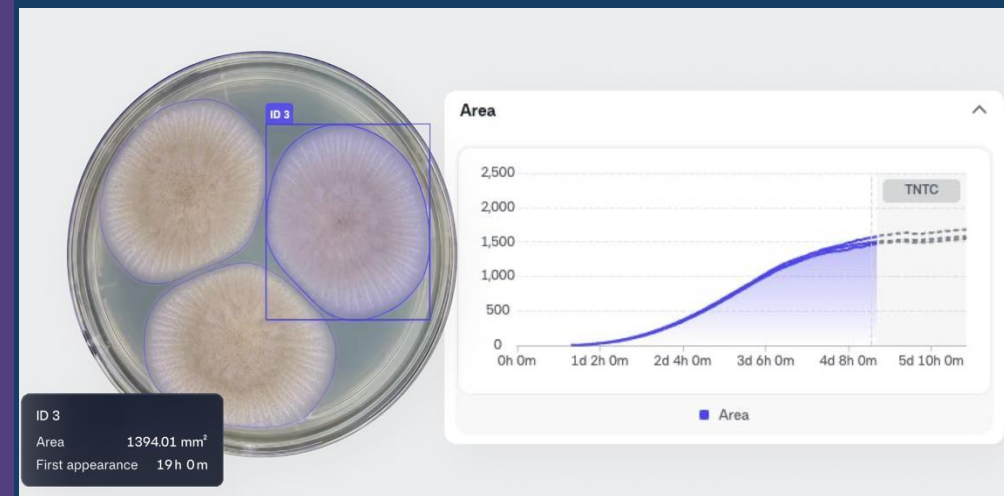
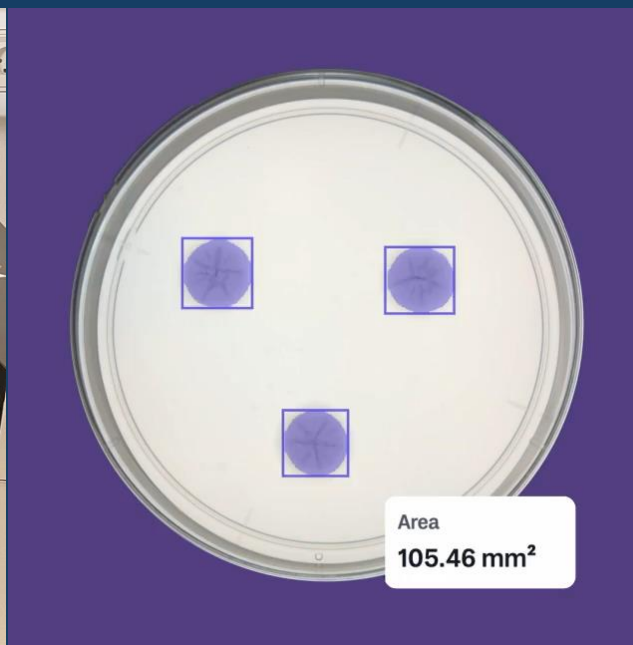


Results: morphological characterization



RESHAPE

advanced laboratory device that combines incubation, imaging, and AI to monitor and analyze microbial growth





Results: morphological characterization



RESHAPE

advanced laboratory device that combines incubation, imaging, and AI to monitor and analyze microbial growth



23°C - 10 days

A. alternata

A. infectoria

A. solani

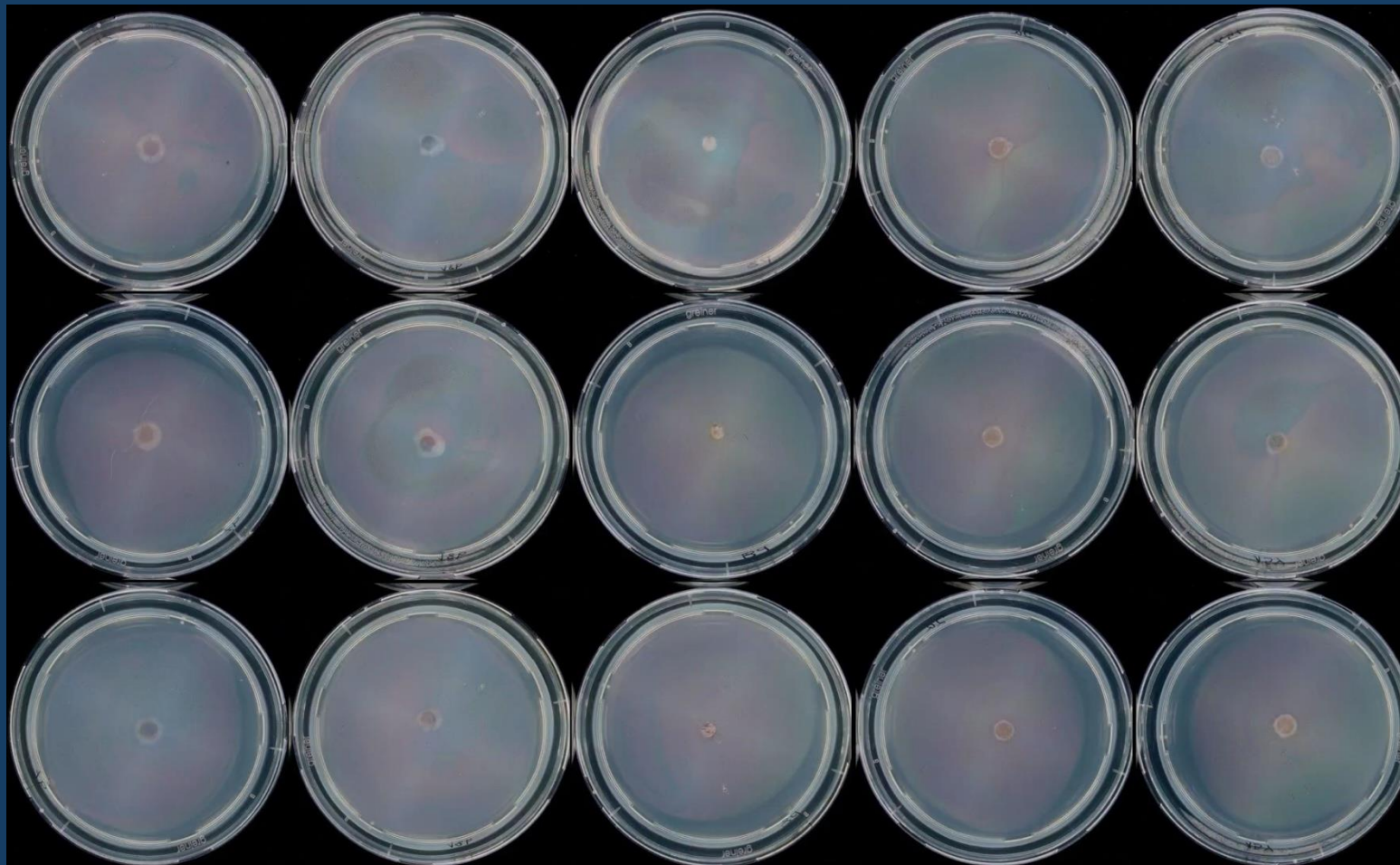
A. protenta



Rep_1

Rep_2

Rep_3



23°C - 10 days

A. alternata

A. infectoria

A. solani

A. protenta

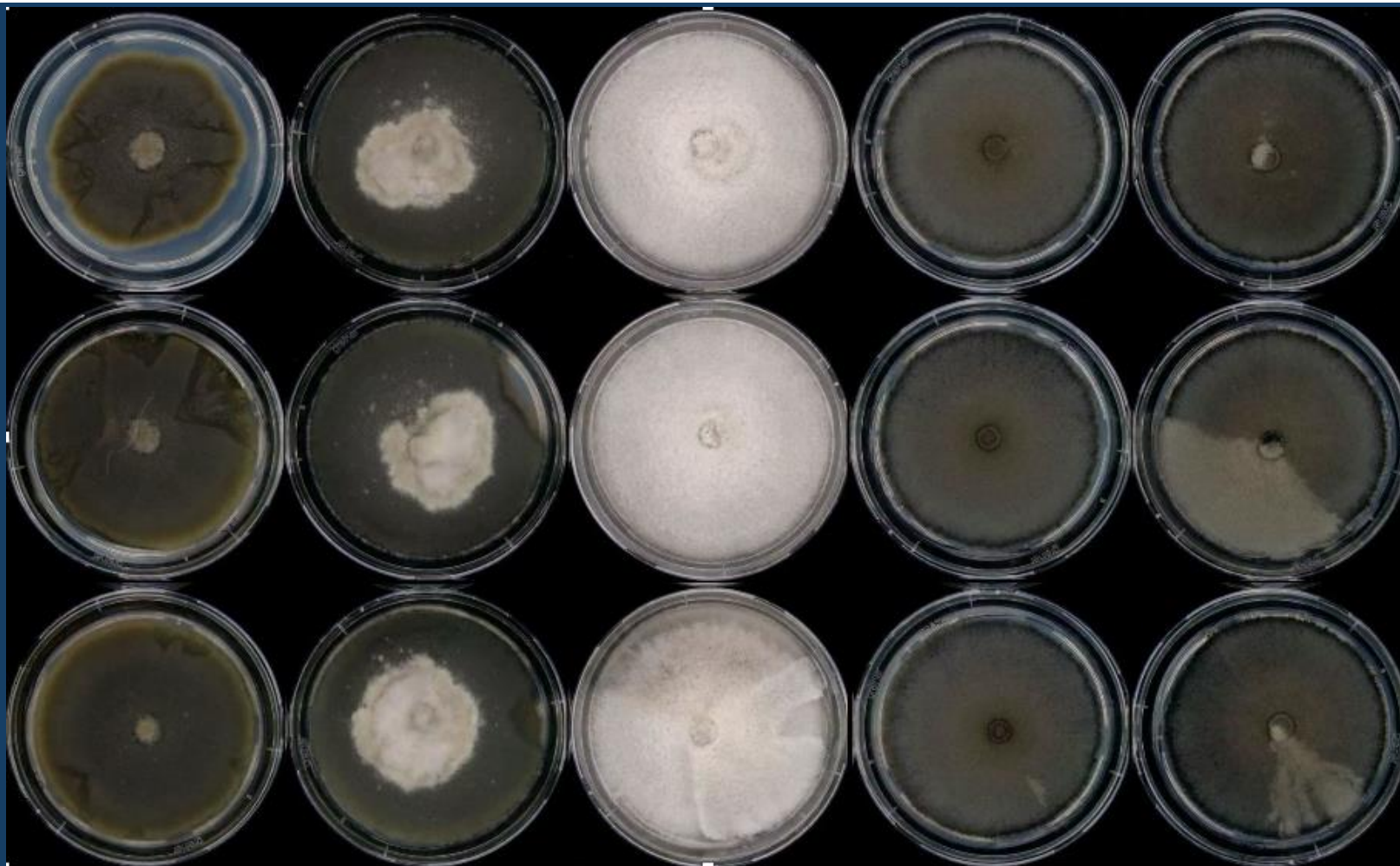




Rep_1

Rep_2

Rep_3



23°C - 10 days

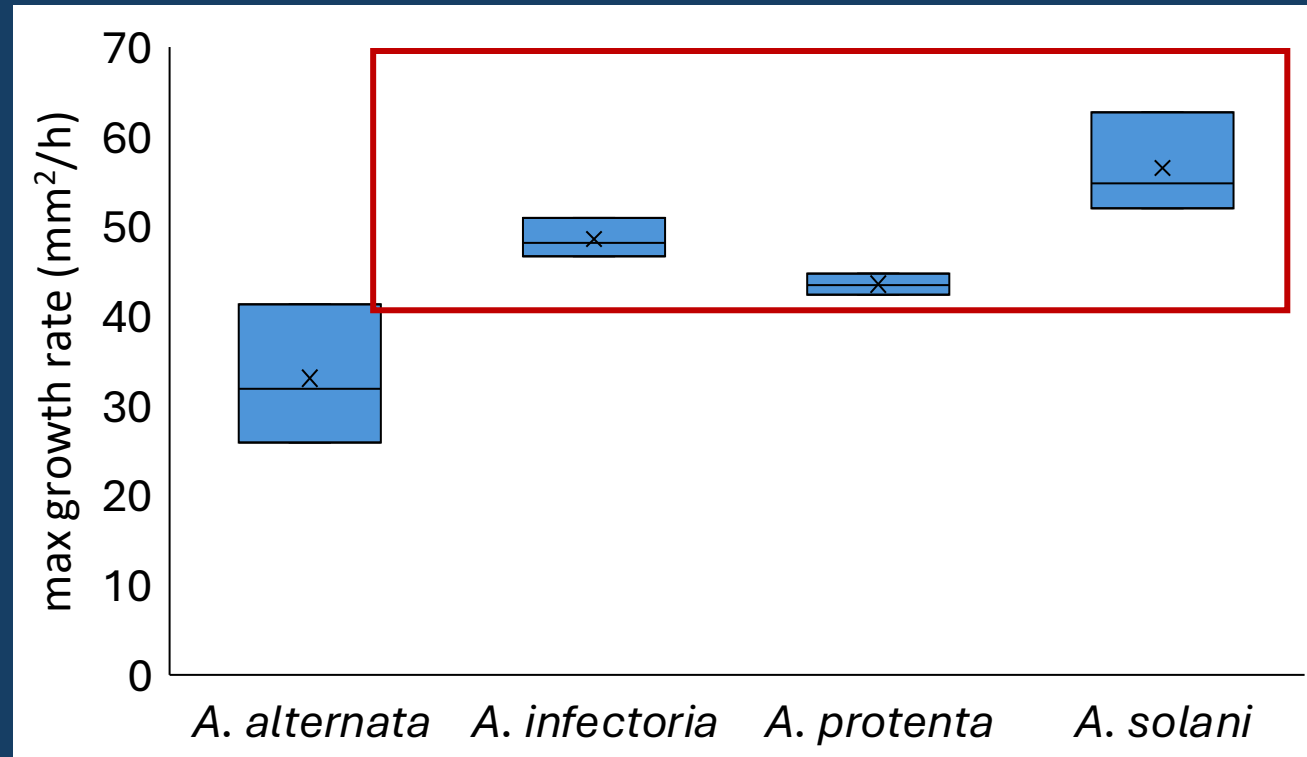
A. alternata

A. infectoria

A. solani

A. protenta

 RESHAPE

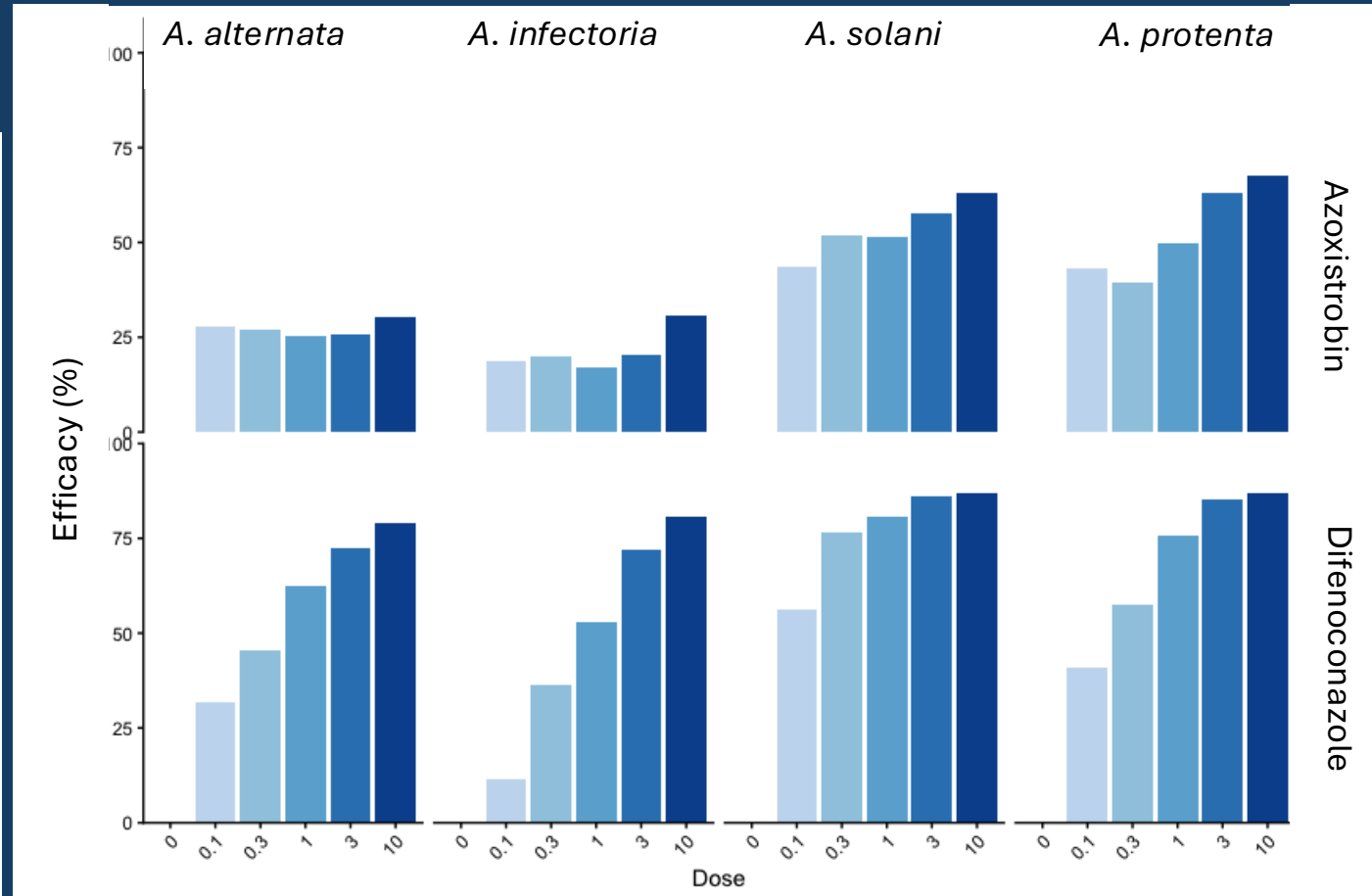
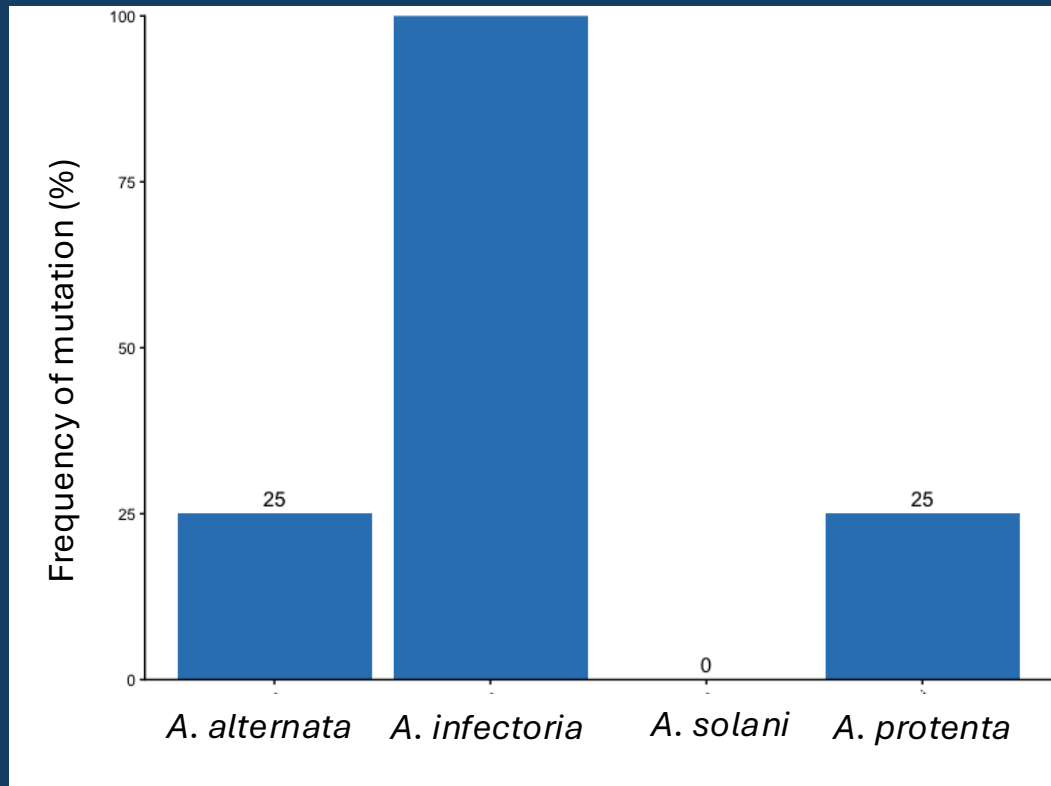


23°C - 10 days



Resistance detection of the G143A Mutation that confers resistance to QoI fungicides in *Alternaria* isolates

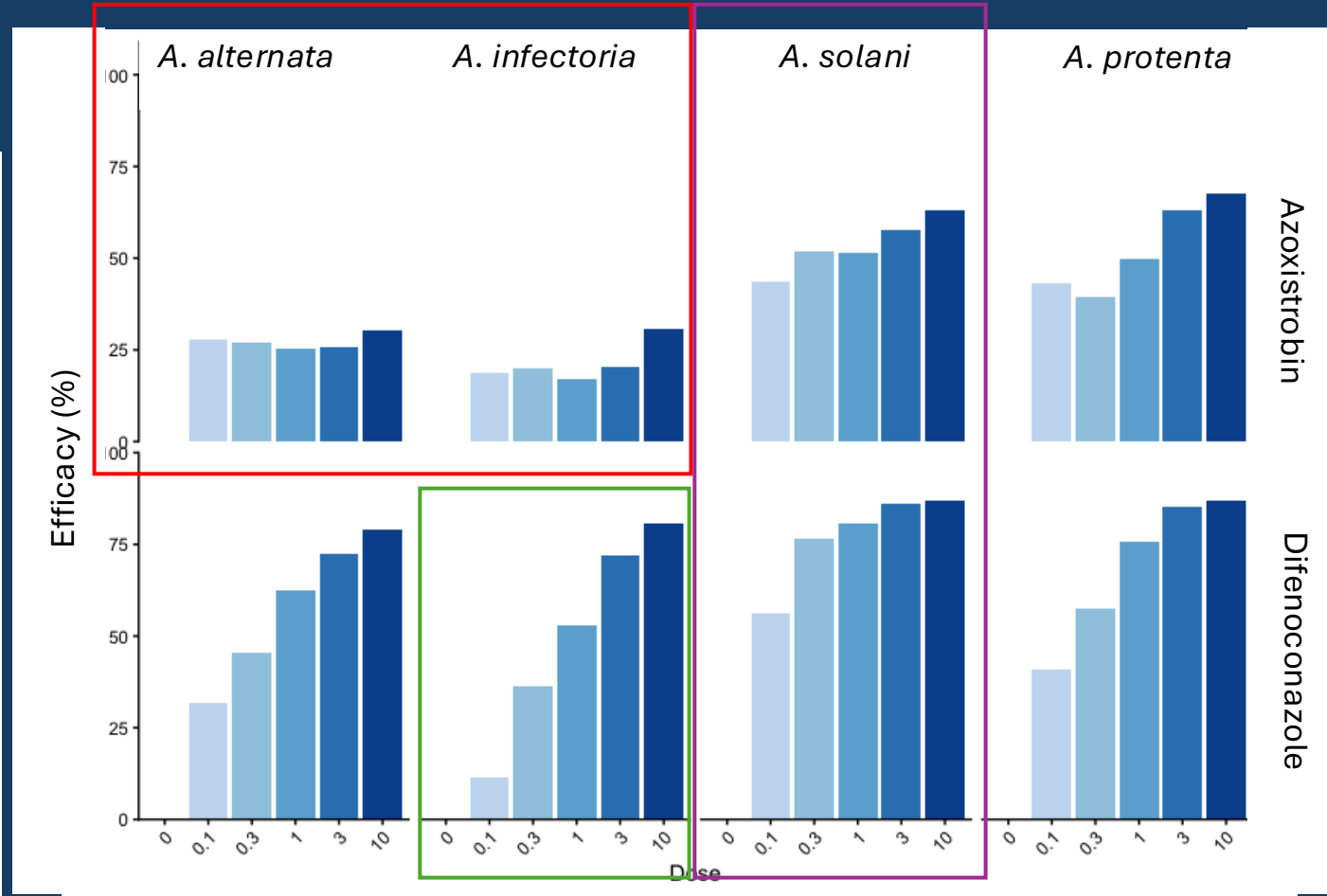
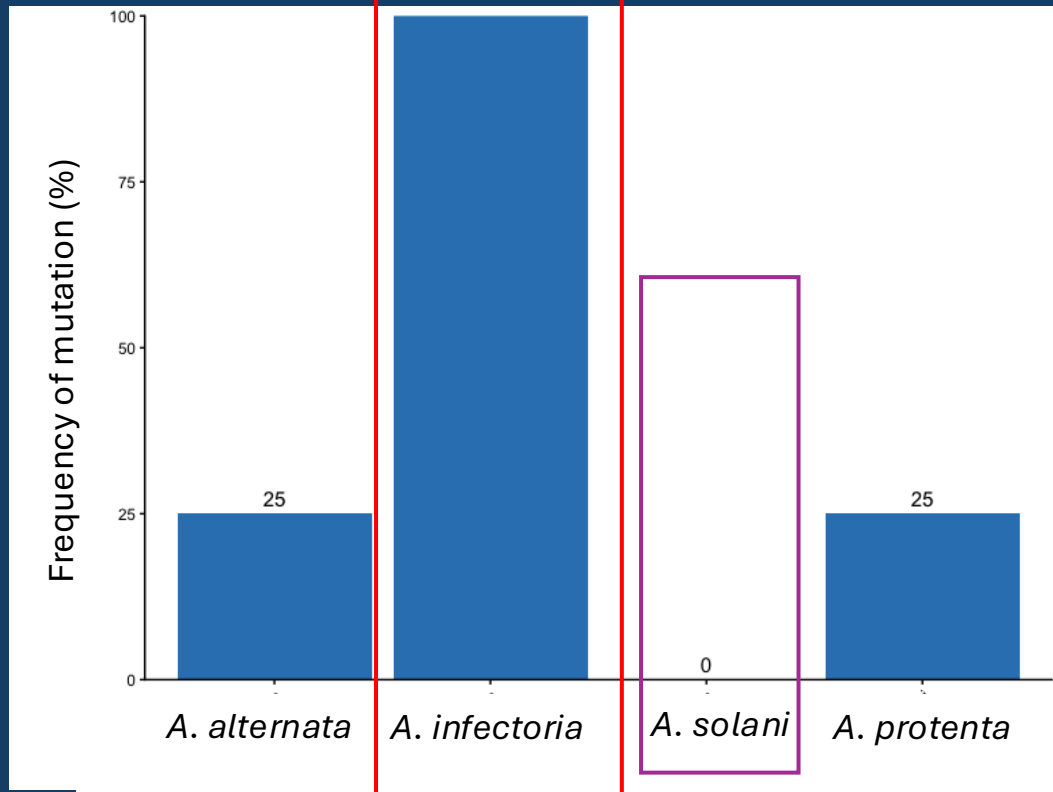
Frequency of the G143A Mutation





Resistance detection of the G143A Mutation that confers resistance to QoI fungicides in *Alternaria* isolates

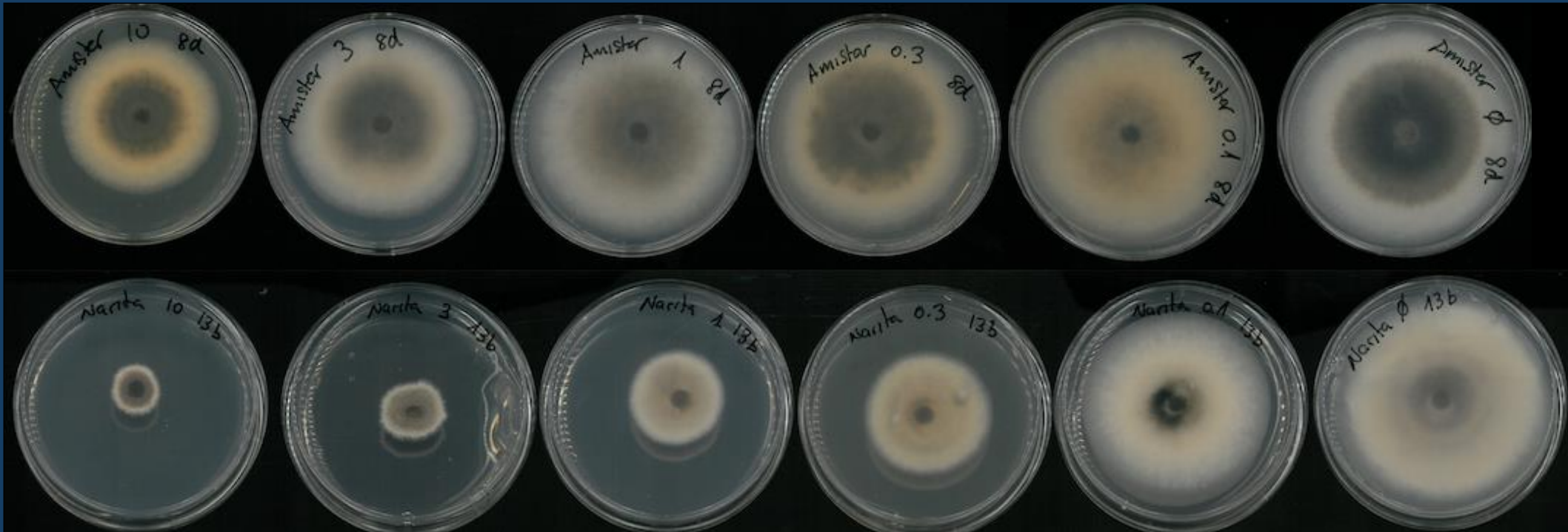
Frequency of the G143A Mutation





Resistance detection of the G143A Mutation that confers resistance to QoI fungicides in *Alternaria* isolates

A. infectoria



Azoxystrobin

Difenoconazole



- **Main conclusions**

1. Potato early blight lesions contained diversity of *Alternaria* species.
2. Strong differences were found between Spain and Denmark.
 1. Spain showed greater species diversity.
 2. Denmark was dominated by *A. protenta*.
 3. Looking forward to know Idaho and Australia sampling results
3. Importance to study diversity of *Alternaria* in different potato producing areas to better understanding
4. Molecular surveillance should be integrated into European monitoring programs and management strategies towards sustainable potato production.



We need to join and research together to design action plans against early blight



Samples from two more areas have been received 😊

We will analyse and incorporate in final results





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Many Thanks

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