An improved PCR method for rapid and accurate identification of mating types in *P. infestans.*

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Mating type, an important phenotypic trait:

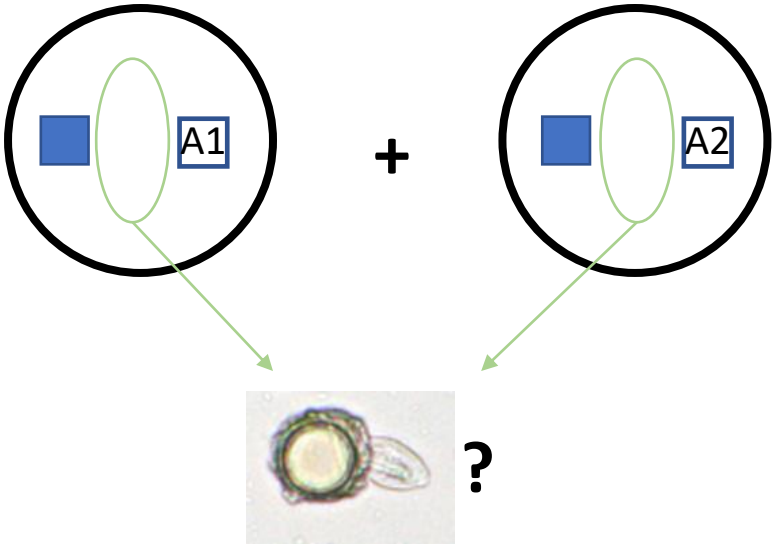
- ***P. infestans* can reproduce in two ways:**
  - ✓ **Asexually** by production of sporangia, only one strain is necessary,
  - ✓ **Sexually** by production of oospores when two strains of opposite mating type, A1 and A2, come in contact.

- ***P. infestans* reproduction impact of late blight population:**
  - ✓ **Asexual** reproduction leads to a rapid disease spread with clonal local populations,
  - ✓ **Sexual** reproduction by contrast generates inoculum with long survival ability and sexual local populations.

Mating type information is very important for populations study and understand *P. infestans* epidemiology.

Tools to determine mating type
1- Pairing test

➢ ***Protocol*:**



✓ On two Petri dishes, the **unknown isolate is confronted to A1 and A2 references isolates**,

✓ **After 10 to 14 days, oospores observations in the dish gives the result :**

* if oospores are observed in the dish with A1 reference, the unknown isolate is A2 and vice versa,

* if oospores are present in both dishes, the isolate is either self-fertile or a mixture of A1 and A2 genotypes.

➢ ***Pros and cons***

- ✓ **Reference mating type technique,**
- ✓ **Very efficient,**
- ✓ **Simple to do,**

- ✓ **Requires live isolates,**
- ✓ **Laborious and long to implement (preparation and waiting time).**

Tools to determine mating type: 2- Molecular tools

➢ ***In the literature, two PCRs are used to determine mating type in one step:***

- ✓ PHYB1/2 by Kim and Lee (2002): **A2 strains** identification,
- ✓ S1a/b by Judelson (1996): **A1 strains** identification.

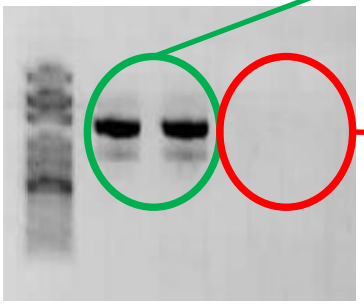
➢ ***Pros and cons***

- ✓ **Fast technique,**
- ✓ **Simple to do,**
- ✓ **Applicable to dead or archived biological samples and FTA cards.**

- PHYB PCR:
  - ✓ 14% of false responses (Brylinska et al., 2018). **DISCARDED**
- *S1 PCR:*
  - ✓ **Highly efficient amplification (96%) (Brylinska et al., 2018)** **SELECTED**
  - ✓ **Gives a result only for the A1 strains.**

How to improve the S1 PCR ?

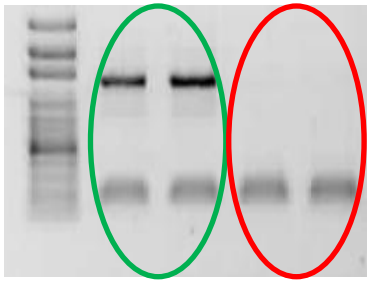
➢ ***S1 PCR results, BUT?***



A1 strains

A2 strains or PCR problems like no DNA or not enough DNA (quantity and quality)

➢ ***Add an internal control can avoid the false A2 determination:***



- **Internal control: PinfTQ primers (Lees, 2012) are generic, and amplify all *P. infestans* strains.**
- **Two distinct profiles of amplification for A1 and A2 strains.**

PCR development:

➢ *PCR conditions :*

- ✓ Parameters tested:
  - Different primers concentrations,
  - Number of PCR cycles,
  - Hybridation temperature PCR.

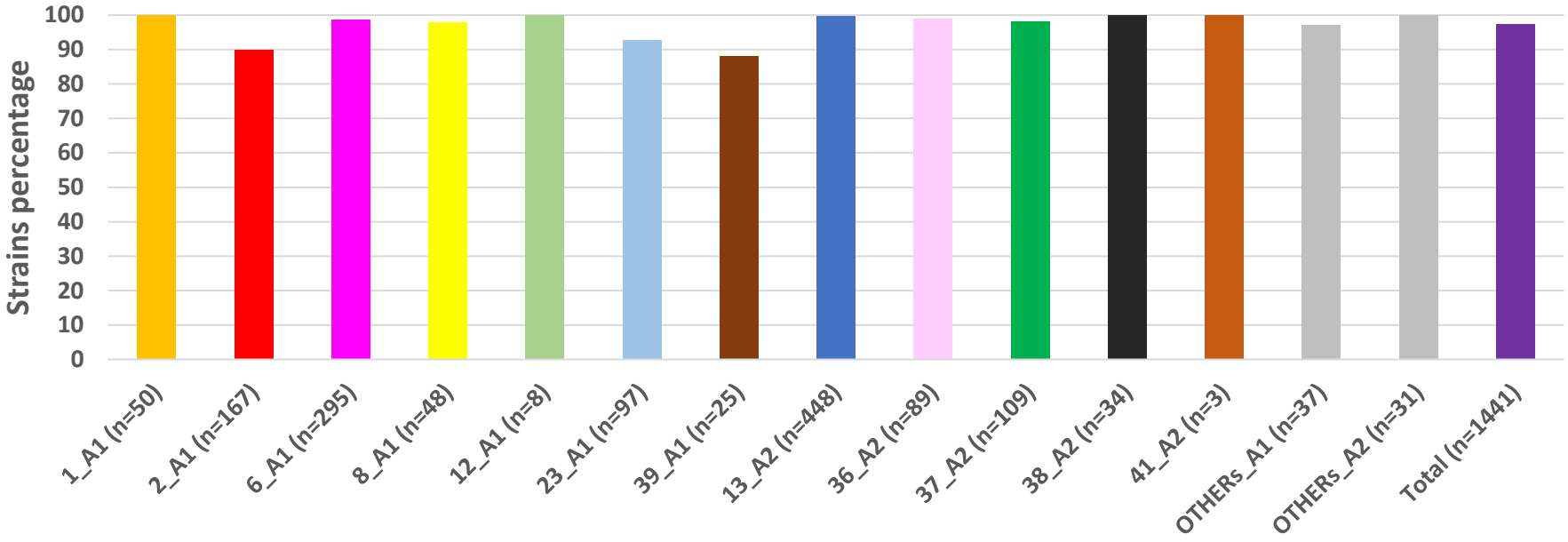
- ✓ Parameters selected :
  - Primers concentrations: S1 at 0.4µM and PinfTQ at 0.05µM,
  - Number of PCR cycles: 35,
  - PCR hybridation temperature : 50°C.

➢ *Validation of multiplex PCR:*

- ✓ Step 1 : 170 samples tested by comparison with the original S1 PCR across five MLLs (EU_2_A1, EU_6_A1, EU_23_A1, EU_13_A2 and EU_38_A2) and some “OTHER” isolates :
  - 100% matches between the PCRs.

- ✓ Step 2 : extended validation with 1441 samples representing all the most European clonal lineages.

Results multiplex PCR:



- **Multiplex PCR matched with pairing test or genotyping results in 97.4 %** of the 1 441 samples.
- **The mating type of most clonal lineages was correctly assigned - between 97 % and 100 %.**
- **98.5 % of correct assignation in "Others" isolates.**

Results:

- **Most mistakes for three lineages:** EU_2_A1, EU_23_A1 and EU_39_A1 with around 90 % of correct determination.

- **Why were some A1 strains (37 samples) identified as A2 with the multiplex PCR?**

  - ✓ Low DNA quality: too long storage,
  - ✓ Low DNA quantity: too long storage and DNA FTA cards,
  - ✓ Efficiency of generic PCR (small amplified fragment) higher than that of S1 PCR.

- **High success rate** of the multiplex PCR for **FTA cards DNA (95%)** and **lyophilised mycelia DNA (98%).**

Conclusion:

- **A very efficient tool to determine *P. infestans* mating type.**
  - ✓ Efficiency equal to that of the S1 original PCR,
  - ✓ Quality of the result assured by the generic PCR internal check.

- **Use high quality DNA**, which can be checked by DNA measurement or by the quality of the SSR profile.

- As explained by Brylinska (2018), **multiplex PCR must be tested in parallel to the pairing test to check its efficiency on the local population** before using the multiplex PCR in routine.

- **Tool suitable for use to analyse mating type from FTA card samples**
  > very useful for the Euroblight community.

Thank you for your attention.

Article with all results:

Mabon R., Guibert M, Corbière R., Andrivon D., 2021.
An improved PCR method for rapid and accurate identification of mating types in the late blight pathogen Phytophthora infestans.
Plant Health Progress 22, 362-367 ; Special issue: Stubborn Oomycetes. https://doi.org/10.1094/PHP-02-21-0026-FI