Cryopreservation of *Alternaria solani* and *Phytophthora infestans*

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INTRODUCTION

Various fungi belonging to all taxonomic groups are routinely used in the disease control institutes of Bayer CropScience. The maintenance and mass production of this large variety of phytopathogenic fungal strains is essential for a high quality biological testing in the fungicide screening process. After an exhaustive comparison of different storage methods, cryopreservation of fungal material in the vapor phase of liquid nitrogen (-160°C) has been chosen to guarantee a reliable delivery of phytopathogenic fungal isolates for routine tests in the lab, greenhouse and field.

While conidia of most fungi (e.g. *Alternaria solani*) are relatively resistant to cellular injury during freezing and thawing, asexual bodies of many *Phytophthora* species (sporangia) are very susceptible to this kind of damage (Fig. 1). The objective of our work was to optimize the cryogenic storage procedure to define conditions that increase viability of *Phytophthora infestans* sporangia for their direct use in the screening process.

MATERIALS AND METHODS

Experiments were performed with sporangia (and zoospores) of 15 different isolates of *P. infestans*. 21 cryoprotectants (penetrating & non-penetrating) in varying concentrations and compositions combined with nine different freezing methods and four different thawing temperatures were used. Viability of sporangia and zoospores were analyzed by germination assays on H_2O agar 14 days and three months after freezing. Disease development after inoculation of tomato plants was assessed to determine the effects of the storage conditions on pathogenicity.

RESULTS

Viability of both sporangia (Fig. 2) and zoospores (data not shown) after storage varies depending on the isolate of *P. infestans*.

Moreover, the viability of *P. infestans* sporangia and zoospores could be significantly improved by the use of both cryoprotectants (Fig. 3) and controlled freezing. The most suitable cryoprotective agents were DMSO (15 %) and propylene glycol (12 %).

The highest recovery rates (45 % for sporangia and 67 % for zoospores) were obtained for samples of *P. infestans* frozen in DMSO (15 %) placed at -20°C for 3h, followed by -80°C for 3h and finally stored in liquid nitrogen (Fig. 4).
Pathogenicity of stored sporangia and zoospores was confirmed in a bioassay with tomato plants (data not shown), even after 3 months of storage at -160°C.

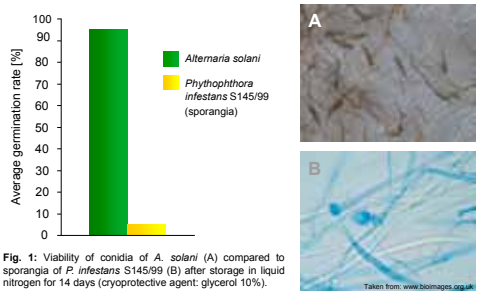

Fig. 1: Viability of conidia of *A. solani* (A) compared to sporangia of *P. infestans* S145/99 (B) after storage in liquid nitrogen for 14 days (cryoprotective agent: glycerol 10%).

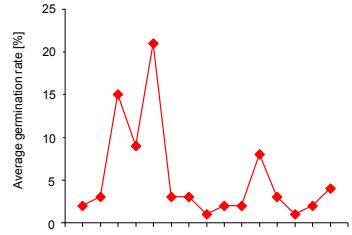

Fig. 2: Influence of different isolates on the viability of sporangia of *P. infestans* after storage in liquid nitrogen for 14 days (cryoprotective agent: glycerol 10%).

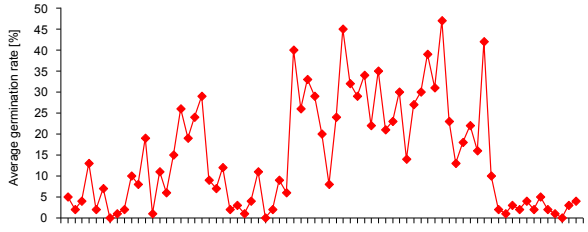

Fig. 3: Effect of different cryoprotective agents and freezing methods on the viability of sporangia of *P. infestans* S145/99 after storage in liquid nitrogen for 14 days.

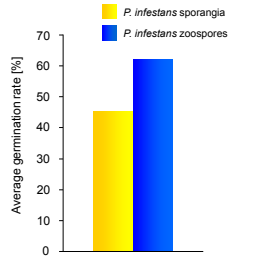

Fig. 4: Viability of sporangia compared to zoospores of *P. infestans* S145/99 after storage in liquid nitrogen for 14 days (cryoprotective agent: DMSO 15%).

CONCLUSION

Phytophthora infestans, the causal agent of late blight, is a problematic pathogen regarding storage due to its sensitive sporangia. The results of this work demonstrate that cryopreservation using DMSO (15 %) and controlled freezing represents an effective method for long-term storage of *P. infestans* inoculum. Cryopreservation thereby offers an unique solution to optimize the time of maintenance and production of the *P. infestans* inoculum used in the different screening tests.

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