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Proteomic analysis of the fungus *Verticillium albo-atrum*

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INTRODUCTION

Verticillium wilt is a vascular disease caused by the soil borne pathogenic fungi *Verticillium albo-atrum* and *Verticillium dahliae. V. albo-atrum* isolates infecting hop display different levels of virulence, resulting in mild or lethal symptoms. Slovene isolates have been determined as PG1 (mild) or PG2 (lethal) pathotype based on pathogenicity tests and AFLP analysis. The spread of the lethal form in hop gardens in the Savinja Valley is a serious threat to hop production. From 1997 to 2006, more than 180 ha of hop gardens were infected and almost 90 ha were destroyed. Due to the lack of effective phytopharmaceutical agents, the only effective means of fighting the disease are phytosanitary and hygienic measures, crop rotation and planting resistant varieties. We employed a proteomic approach to gaining a wide insight into the infection process and to identifying proteins related to infection. This allows simultaneous analysis of total proteins on a two-dimensional polyacrylamide gel.

Fig. 1: Dark resting mycelium of *Verticillium albo-atrum*

Fig. 2: Symptoms of Verticillium wilt on hop plants: browning of the vascular tissue (left) and wilting of the entire plant (right)

MATERIALS AND METHODS

Verticillium albo-atrum was grown in a general fungal medium for 1 week and mycelium was collected by filtration. Proteins were extracted according to Jamnik et al. and the concentration was measured with a Bradford reagent. 450 µg of proteins were precipitated, resuspended in rehydration solution and applied to 13 cm IPG strips (pH 4-7) by rehydration loading. After isoelectric focusing, strips were equilibrated and loaded on 12.5% polyacrylamide gels. After electrophoresis, gels were stained with Coomassie Brilliant Blue G-250, as described by Neuhoff et al. and scanned. Dymension 2 software was used for image analysis and statistical tests. Four biological and three technical replicates were included in the experiment (a total of 24 gels).

RESULTS

Separation of proteins was good and there were only a few spots with horizontal or vertical streaks. Reproducibility between technical replicates was excellent, while biological replicates displayed minor differences. On average, 2645 spots were detected in PG1 (660 valid) and 2529 spots in PG2 (721 valid). A spot was determined as valid if it was present on at least 9 replicate gels (out of 12) and if it passed filtering criteria. There were 173 spots present only in the PG1 pathotype and 234 spots were found only in the PG2 pathotype; 487 spots were observed in both pathotypes. A regulation factor of 2 was set, which resulted in 64 spots significantly more abundant in the PG1 pathotype and 90 spots significantly more abundant in the PG2 pathotype ($p<0,05$). Twenty-seven spots were chosen for subsequent MS analysis.

CONCLUSION

Two-dimensional electrophoresis was used successfully for the separation of cellular proteins from *Verticillium albo-atrum*. Significant differences between PG1 and PG2 pathotypes were observed and will be further investigated. Furthermore, the general fungal medium will be replaced by a simulated xylem fluid medium (SXM) to induce the expression of genes related directly to infection.

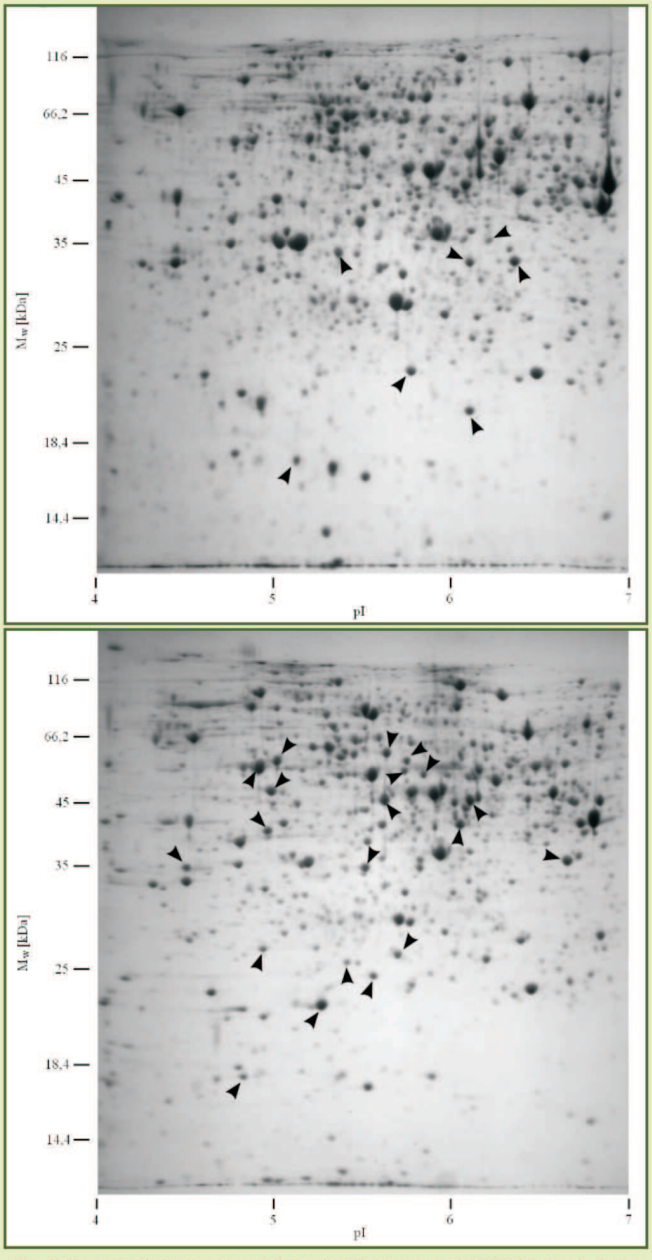

Fig. 3: Representative images of 2-D gels: PG1 (upper) and PG2 (bottom). Spots chosen for MS analysis are marked by arrows.

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