



## **PHYTOPHTHORA SPECIES IN FOREST TREES IN HUNGARY – A GENETIC APPROACH**

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Disease symptoms caused by different *Phytophthora* species were studied in Hungary since 1999. A collection of more than 300 cultures from 6 main localities and from four different hosts (*Alnus glutinosa*, *Juglans nigra*, *Quercus petraea* and *Q. cerris*) was established. The identification of the isolates presented sometimes difficulties because of the absence of adequate morphological characters. In 2006 we have started to use molecular markers for species identification based on the methods and results published on the identification of different *Phytophthora* species and the advances of the available molecular methods at our institute. Altogether 80 isolates were analyzed. DNA extraction and PCR assay were performed using the REDExtract-N-Amp kit from Sigma. The amplification of the DNA was carried out followed the manufacturer's instruction with the primers "ITS4" and „ITS6". Initial denaturation step of 3 min at 94° C, followed by 35 cycles of 94° C (30 sec), 55° C (30 sec) and 72° C (90 sec) and a final extension step at 72° C (10 min). PCR was performed in an Eppendorf Mastercycler and the sequence reactions on an AB 3730XL automatic sequencer respectively.

The DNA sequence was alignment by ClustalX using default settings. Distance analysis was performed by the Neighbor Joining NJ algorithm. The distance matrix was calculated based on the Tamura-Nei substitution model, while the robustness of the topology was tested by bootstrapping with 1000 repetitions. For comparison also several GenBank (<http://www.ncbi.nlm.nih.gov/>) entries of different *Phytophthora* taxa were taken.

74% of the amplified DNA resulted in sequences (59 isolates). Altogether seven already described and at least three undescribed (or at least in the GenBank as *Phytophthora* sp. documented) *Phytophthora* taxa were found. Eight of them were found in *Alnus* (*P. alni*, *P. citricola*, *P. gonapodyides*, *P. inundata*, *P. megasperma* and *P. sp1*, *P. sp2*, *P. sp3*), four in *Juglans* (*P. cactorum*, *P. citricola*, *P. hedraiaandra*, *P. sp1*) and two in *Quercus* (*P. citricola*, *P. gonapodyides*). The sequences of the Hungarian isolates represents 4 major clades: 1: *P. inflata/citricola*, 2: *P. cactorum/ideae/hedraiaandra*, 3: *P. inundata/megasperma/gonapodyides* and 4: *P. alni*. The mostly widespread is the *P. citricola/inflata* group, having four haplotypes (17 isolates) from all four investigated hosts. *P. gonapodyides* showed the highest polymorphism, having 7 haplotypes (11 isolates) on two hosts (*Alnus glutinosa* and *Quercus petraea*).

Three undefined taxa (*P. sp1*, *sp2*, *sp3*) didn't result in an exact species determination even after an intensive BLAST search in the GenBank. All haplotypes found were submitted to the GenBank and are under revision at the time of abstract submission.

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