the SPS is to ensure that phytosanitary measures on trade are applied only for plant health and are based on scientific principles. For harmonization with the SPS, the Plant Protection Act of Korea was revised in 1995. Under the act, the National Plant Quarantine Service (NPQS) has conducted quarantine inspection on all imported plants and plant products at the international ports to prevent the introduction of exotic pests. The NPQS also inspects agricultural products for export to meet the requirements of the importing countries.

When quarantine pathogens are detected from the agricultural products during the inspections, appropriate actions including destruction, re-shipment or treatment are taken. Quarantine pest is defined as a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled. More than 300 shipments of agricultural products were destroyed or re-shipped in 2003 in Korea, because they were infested by quarantine pathogens including Arabis mosaic virus.

In order to support science-based plant quarantine activities, 13 researchers including myself are responsible for conducting research and teaching port inspectors. Our research fields are mainly focused on:

- Development of detection and identification methods for plant quarantine pests
- Study of distribution, ecology and management options of plant pests
- Development of treatment methods for importing and exporting plants

Seeds can be efficient means of moving pathogens between geographical regions (McGee, 1997). Therefore I carried out the project on the identification of seed-borne fungi having quarantine significance from 2001 to 2003. Using the results, the identification manual was published for port identifiers this year.

In summary, plant quarantines are important control methods that exclude exotic pathogens. For the best control effect, it should be based on scientific principles.

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SIII-3

Molecular taxonomy and speciation in *Pythium* and *Phytophthora*

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Pythium and *Phytophthora* are genera of the algae-like Oomycetes in the Straminipile clade of the kingdom Chromista . They comprise many plant pathogens with an important impact on agriculture and forestry including some species among *Phytophthora* that have caused very severe outbreaks. Classic taxonomy is based on morphology. Unfortunately, characters often vary among isolates of the same species, and show significant overlap among species. Therefore, species delimitation and identification is notoriously difficult. Molecular methods may overcome these problems and are increasingly being used for identification and phylogenetic studies.

Pythium: phylogeny, species delimitation and detection

In a study of *Pythium*, we used a variety of molecular methods to study more than 500 isolates, representing all species presently available in pure culture (approx. 100). One representative isolate of each species was used for sequencing of the ribosomal internal transcribed spacer (ITS): all ex-type, neo-type, authentic or otherwise well defined representative strains. For half of the species, the D1-D3 regions of the large subunit

(LSU) DNA was sequenced for comparison with ITS results. All strains were used in restriction analyses of PCR amplified ribosomal intergenic spacer (IGS) and ITS and in studies of random amplified polymorphc DNA (RAPD) patterns, to reveal infraspecific variation and to establish species boundaries.

Parsimony analysis of the *Pythium* ITS-sequences (Lévesque & de Cock, 2004) showed the same trends as a combined phylogenetic analysis of the LSU D1-D3 region for *Pythium* and *Phytophthora* (Fig. 1). Two major clades were generated, one representing the *Pythium* species with filamentous sporangia (subclades A-C) and the other one with globose sporangia (subclades E-K). A small clade (D) was found in between the two main clades, comprising species with either contiguous sporangia or contiguous hyphal swellings. Most species with filamentous, inflated sporangia clustered in a single subclade, separated from those with non-inflated sporangia. Species with globose, proliferating sporangia were present in four subclades (E, G, H, K), however, all of these clades also contained species with non-proliferating sporangia. Species producing hyphal swellings but no zoospores all clustered in the globose sporangium clade, with a single exception.

A total number of eleven smaller clades (A-K: Fig. 1) was recognized, which often correlated with host-type or substrate and in some cases with few morphological characters (e.g. ornamented oogonia). Most characters used in *Pythium* taxonomy, such as position and number of antheridia, oospore type etc., did not or only indistinctly correlate with phylogeny. It is important to point out that clade K which contains *P. vexans* is more closely related to *Phytophthora* and would probably deserve a new genus name as suggested by others in previous studies.

Infraspecific variation was studied by a comparison of the ITS sequences of the ex-type and representative strains with all ITS sequences of *Pythium* in GenBank. A total of 26 species, of which a number has been recently described, had ITS sequences identical or nearly identical to formerly described species, suggesting possible conspecificity. Conspecificity could be confirmed by RAPD and RFLP analysis for a number of species. More than 30 species proved to be heterogeneous. In a number of cases this was apparently due to misidentifications, however, in many other cases differences were sufficiently solid to justify new species descriptions.

ITS sequences were also used for development of species-specific oligonucleotides. The latter were used in DNA-arrays for simultaneous detection and identification of multiple pathogens in environmental samples.

Phytophthora: interspecific hybridization and speciation

When compared to a large sampling of *Pythium* species, *Phytophthora* is monophyletic, except for one marine species, whereas *Pythium* is not because of clade K (Fig. 1). Kroon *et al.* (2004) noticed slight differences between the topologies of a phylogenetic tree based on nuclear DNA sequences and a tree based on mitochondrial DNA sequences. They assigned this to possible hybridization events in the past, suggesting that reticulation may play a part in speciation in *Phytophthora*. A study using dimeric isozymes and nuclear as well as mitochondrial DNA sequences, demonstrated that hybridization between *Phytophthora nicotianae* and *Phytophthora cactorum* has occurred in nature (Man in 't Veld *et al.*, 1998). These two species cluster in the same clade in phylogenetic trees, however, they are present in different subclades (Kroon *et al.* 2004). Isozyme studies of a group of morphologically unidentifiable isolates revealed heterozygosity at certain loci for a number of them, which may point the a hybrid nature.

Another feature playing a part in speciation in *Phytophthora* is host specificity. Strains morphologically identified as *Phytophthora porri* have been isolated from *Allium porrum*, *Brassicae* spp. and some other hosts. A study of pathogenicity, morphology, DNA and isozymes showed that isolates from *A. porrum* resp. *Brassica* spp. are in fact two different, host specific species, of which the isolates from the latter host have now been described as *P. brassicae* (De Cock *et al.* , 1992; Man in 't Veld *et al.* , 2002). Preliminary results of our continuing research in the *P. porri* complex revealed that isolates of *P. porri* from hosts other than *Allium* or *Brassica* are different, possibly representing new species. Restriction analysis and sequencing showed in three isolates of *P. porri* from *Allium* spp. the presence of ITS sequences of both *P. porri* and *P. primulae*; they are presumed to be hybrids between those two species. *P. primulae* was shown to be a host specific species too; isolates identified as *P. syringae*, isolated from *Primula* spp. all turned out to be identical to *P. primulae*.

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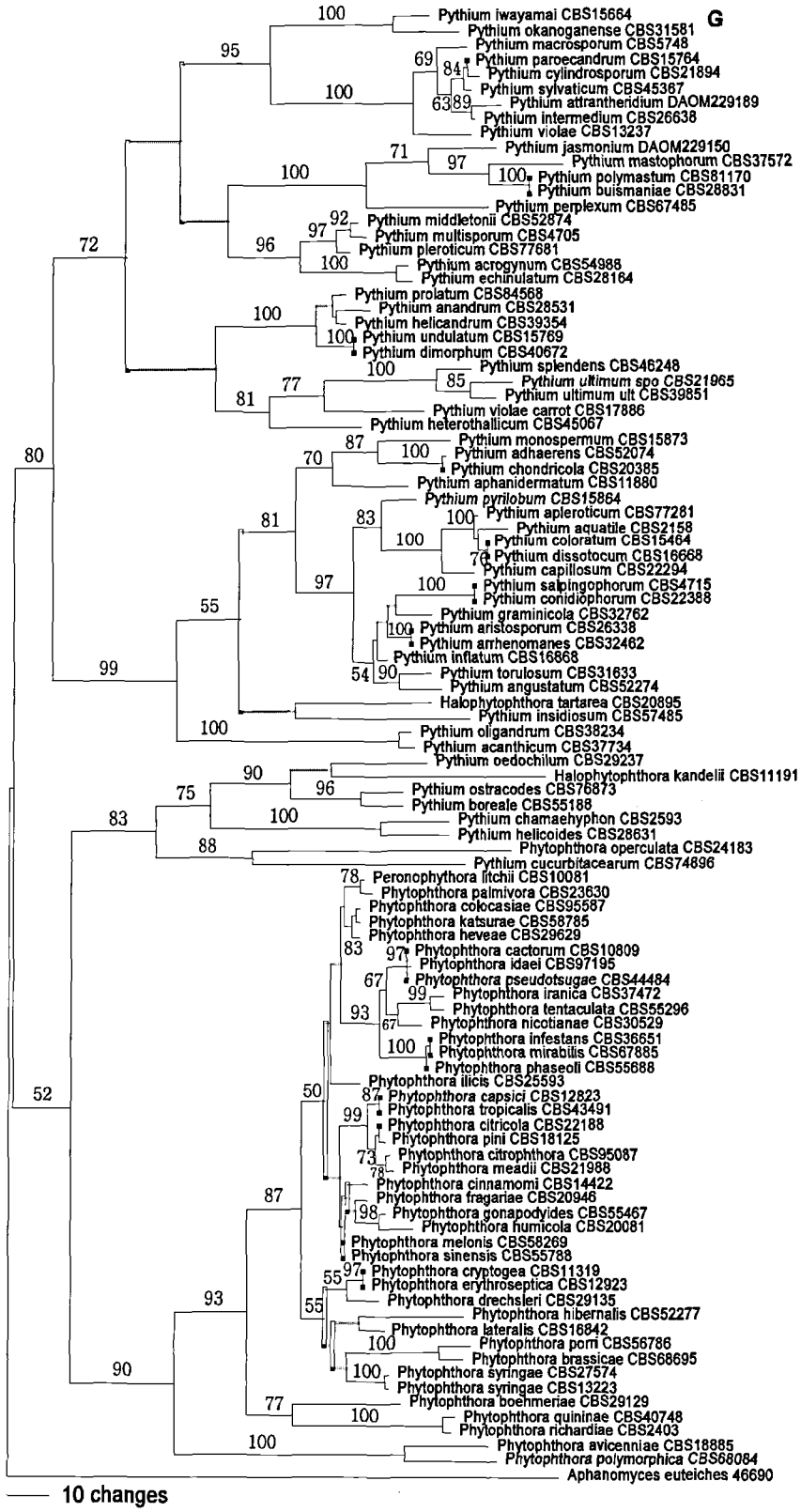

Fig. 1. Phylogeny of *Pythium* and *Phytophthora* species based on the D1 to D3 regions of the rDNA LSU. First of the 2157 equally parsimonious trees of a heuristic search is shown. Numbers within the tree represent the bootstrap values (100 replications) and branches that had less than 50% support are grayed out and show no bootstrap value. Length=2039, consistency index = 0.37, rescaled consistency index = 0.30, and retention index = 0.81. Letters and numbers at accolades refer to clades recognized by Lévesque & De Cock (2004) and Kroon *et al.* (2004) respectively.