Report over work done at CBS June-September 2003

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Project: Investigation on fungicolous species of the genus *Verticillium* using morphology and molecules

Three tasks were tackled during the period 26 June-23 September at the Centraalbureau voor Schimmelcultures in collaboration with Prof. Walter Gams.

1. Completion of a set of fungal description sheets as set 158 for ***IMI Descriptions of Fungi and Bacteria*** that will be published in January 2004 by CABI Bioscience. Fungal species described are: *Lecanicillium lecanii*, *L. muscarium*, *L. longisporum*, *L. psalliotae*, *L. aphanocladii*, *L. dimorphum*, *Pochonia chlamydosporia* (varieties *chalmydosporia* and *catenulata*), *P. suchlasporia* (varieties *suchlasporia* and *catenata*), *Haptocillium balanoides* and *Drechmeria coniospora* (in collaboration with Walter Gams).

2. Completion of a paper entitled "**The type species of *Verticillium* and its purported teleomorph, *Nectria inventa***" submitted for publication in Mycological Research (in collaboration with Walter Gams and Hans-Josef Schroers).

3. Continuation of a project entitled "Investigation on fungicolous species of the genus *Verticillium* using morphology and molecules" that was started September 2002 in collaboration with Walter Gams.

The project was started by reviving freeze-dried fungal strains from CBS. Some 150 *Verticillium* and verticillium-like isolates preserved at CBS and some new Iranian isolates were studied using classical and molecular approaches in order to sort out the remaining taxa in *Verticillium* sections *Albo-erecta*, *Prostrata* and the residual group. The cultures were microscopically examined for purity. Total DNA was extracted using Fast DNA Prep Kit for all isolates. In addition to temperature experiments on malt extract agar (MEA) at seven temperatures (15, 18, 21, 24, 27, 30 and 33°C) and growth rate and colony morphology on potato dextrose agar (PDA), camera-lucida drawings and photographs we obtained for each isolate. Taxonomically informative structures are being measured.. Sequences of some 130 representative isolates were obtained for the ITS regions and the 5.8S gene. The phylogeny of these sequences was reconstructed using Neighbor-Joining in PAUP. From the ITS tree at least five distinct clusters of isolates could be identified. These are: 1. Fungicolous isolates around

Verticillium fungicola, 2. Conidial chain-forming isolates under *Verticillium leptobactrum* and *V. insectorum*, 3. Mycoparasites of *Rhizoctonia solani* under *V. biguttatum*, 4. Myxomyceticolous species under *V. rexianum*, 5. A large number of other isolates preserved at CBS under *Verticillium* sp. with oval conidia and undulate phialide tips. *Verticillium fungicola* and its varieties are found closest to the genus *Lecanicillium* in the Clavicipitaceae, a genus that already accommodates fungicolous and entomogenous species. Two taxa are identified in the *V. fungicola* complex based on temperature maximum for growth. *V. leptobactrum* may possibly be accommodated in the newly defined genus *Isaria*. The other three clades are currently being studied and probably three new genera should be described to accommodate these taxa. Species delimitation is currently being studied for each cluster.

In order to obtain the necessary support for the ITS clades, a 500 bp region of the small subunit ribosomal DNA of some 40 isolates representing each clade was sequenced using the primer pair NS1 & Oli1. Phylogenetic analysis of this region though less resolved, supports the clades obtained using ITS sequences. In conjunction with sequences documented in GenBank, teleomorph connections will be elucidated from this material as far as possible. Besides these major clades some more isolated verticillium-like anamorphs remain, which cannot yet be adequately reclassified.

This project has already led to some conclusions and the remaining parts of the work will be completed in the next year jointly at the CBS and PPDRI (Plant Pests & Diseases Research Institute, Tehran, Iran). An oral presentation on the progress of the above project was given at the CBS. The results of the work will be published in due course.