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Establishment of a PCR-RFLP Library for Basidiomycetes, Ascomycetes and their Ectomycorrhizae on *Picea abies* (L.) Karst.

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K e y w o r d s : PCR-ITS-RFLP, digestion pattern library, types of ectomycorrhizae, Norway spruce.

S u m m a r y

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The internal transcribed spacer (ITS) regions in ribosomal genes are variable enough to distinguish most mycorrhizal and other higher fungi to the species level. Polymerase chain reaction in combination with restriction fragment length polymorphism (PCR-RFLP) can be used to establish the digestion pattern library based on fungal sporocarps. We established the digestion pattern library for over 150 spp of mycorrhizal and nonmycorrhizal higher fungi predominantly collected in a four year period on a 1 ha permanent forest research plot of the Slovenian Forestry Institute in an autochthonous altimontane Norway spruce forest on Pokljuka (Triglav national park, 1200m above see level). Only the problematic of genus *Russula* is presented here. The same method is being used on mycorrhizal root tips from the same plot for determination of types of ectomycorrhizae.

I n t r o d u c t i o n

Traditional method for determinaton of types of ectomycorrhizae involve microscopic determination of mycorrhizal root tips (AGERER 1987-1999). This method has some disadvantages (AGERER & al. 1996) which can easily be avoided using PCR-RFLP technique with primers specific for Basidiomycetes and Ascomycetes directly on mycorrhizal root tips (GARDES & BRUNS 1993). Species of the

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fungal symbiont can be determined by comparing the digestion pattern of the mycorrhizal morphotype with PCR-RFLP pattern of previously determined sporocarps. In case PCR-RFLP method fails, sequencing is needed. Only the problematic of the genus *Russula* is presented here.

Material and Methods

Most herbarium specimen used were collected on the permanent forest research plot of the Slovenian Forestry Institute on Pokljuka and some additional fresh specimen were collected on different locations in central Slovenia. Types of ectomycorrhizae were determined on root samples from the same location as described in TROST & al. 1999. All fruitbody exicates and mycorrhizal types (in ethanol) are kept in the herbarium of Slovenian Forestry Institute. DNA extraction and PCR amplification from herbarium specimens, fresh samples and fresh mycorrhizal root tips was performed according to KRAIGHER & al. 1995 as modified by GREBENC & al. 1999. The fragments were amplified by using primer pairs ITS1f-ITS4b (GARDES & BRUNS 1993) for Basidiomycetes and ITS1-ITS4 (WHITE & al. 1990) for Ascomycetes. Restriction digestion of 10µl of PCR products were performed with two units of restriction enzyme (HinfI -5'..G/ANTC..3', TaqI -5'..T/CGA..3' and MboI –5'../GATC..3') as proposed by KÅRÉN & al. 1997. Digested DNA fragments were size-fractioned on 2% LE agarose for 185 min at 140V in 0.5X Tris-borate-EDTA buffer and visualised by ethidium bromide staining. Gels were scanned and analysed with Taxotron® software (Pasteur Institute 1998, Paris, France). In Taxotron® package we have used Restrictoscan® to record the migration of fragments from digitised image, Restrictotyper® to calculate molecular weights of fragments (method by Schaffer & Sederoff, cit. in Taxotron manual), to compare different samples and construct distance matrix, Adanson® to combine distance matrix of all three endonucleases using single-linkage analysis and Dendrograf® to produce a tree.

Results and Discussion

Amplification of ITS regions including 5.8S ribosomal gene were successful for most herbarium and fresh fruit bodies and 199 RFLP patterns from over 150 different species of mycorrhizal and nonmycorrhizal fungi are stored and ready to compare with RFLP patterns from mycorrhizal root tips. The same database was used to draw a tree-description (dendrogram) for the combined single-linkage analysis of RFLP patterns using Dendrograf® software (not shown).

In figure 1 dendrograf and the restriction patterns only for samples corresponding to genus *Russula* are shown. PCR-ITS-RFLP method with three endonucleases (HinfI, TaqI and MboI) was discriminative enough to distinguish all samples of *Russula* spp. *Russula emetica* and *R. emetica* var. *emetica* have closely related ITS region but can still be distinguished by TaqI endonuclease eventhought some more samples should be used to confirm the obtained result.

The sporocarps of four samples, corresponding to *Russula postiana* and *R. subcompacta* (Fig. 1), which were morphologically very difficult to distinguish showed clearly different PCR-RFLP patterns.

Different PCR-RFLP patterns of ITS region within one species might be due intraspecific variation similarly to observed data on the *Hydnum repandum* and *H. rufescens* from different locations in central Slovenia (data not shown and

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AGERER & al. 1996).

For identification of fungal symbiont in ectomycorrhizae the root tips and sporocarps should be collected from the same plot to minimise the possibility of false negative or false positive determinations due to the intraspecific variation.

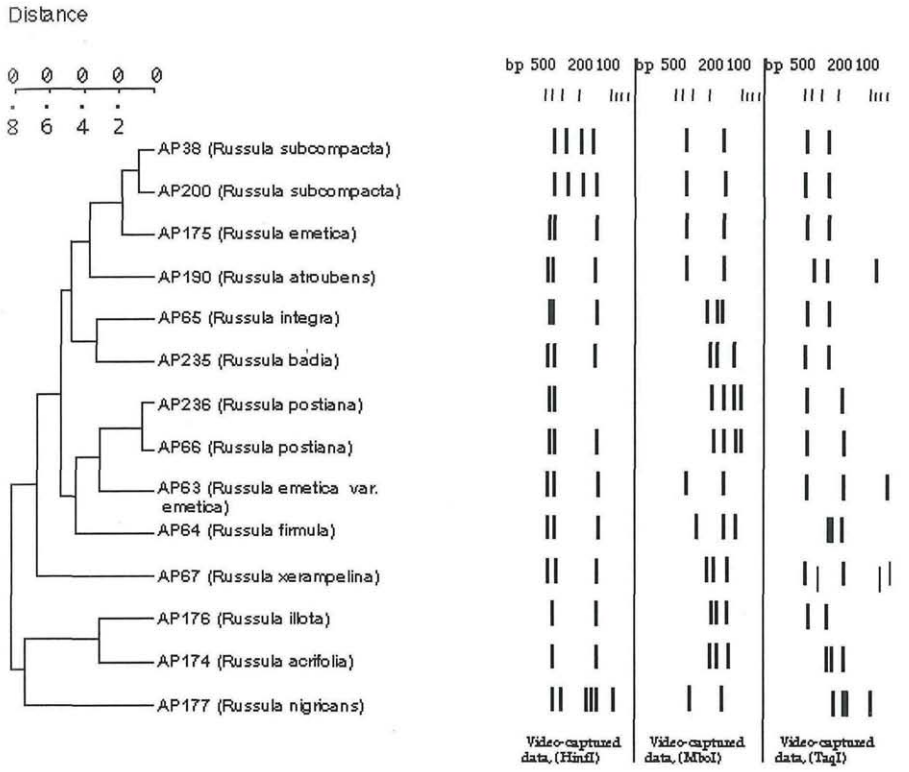

Fig. 1. Tree-diagram of the combined single-linkage analysis of the PCR-RFLP pattern from digestion of the ITS region of rDNA amplified from sporocarps of *Russula* spp. Samples and restriction patterns of *Russula* spp. ITS region obtained by HinfI, TaqI and MboI endonucleases are shown.

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