Application of PCR fingerprinting using (GACA)4 primer in the rapid discrimination of *Trichophyton mentagrophytes* strains isolated from hair from fox fur farming

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Dermatophytes are keratinolytic fungi causing a wide variety of skin diseases, mainly nails and hair of human and animals (ringworm). Their identification is often time consuming due to the morphological intraspecies similarity. The identification of fungi also requires a scientific knowledge and experience. Including molecular methods may be helpful in this procedure.

The aim of this study was to confirm the utility for differentiation of *T. mentagrophytes* variants of dermatophytes strains isolated from fox`s hair from one fur farming (Polish strain with white neck of *Vulpes vulpes*).

Fig. 1 One of examined foxes (*Vulpes vulpes*).

Materials and methods: The specimens of hairs and scrubs were obtained from animals from fox fur farming Batorówka in Moszczenica at the end of February 2014. Hairs were collected by brush technique from 75 randomly selected foxes of Polish strain with white neck (Vulpes vulpes) In this study for confirmation results obtained on conventional way of diagnostics we applied a molecular biology methods: PCR amplification with pan-dermatophyte primers to confirm the presence of dermatophytes DNA and sequencing of the ITS region as a confirmation of identification. After confirmation, another one-step PCR-based approach employing the simple repetitive oligonucleotide $(GACA)_4$ was performed to detect the intraspecies difference among isolates.

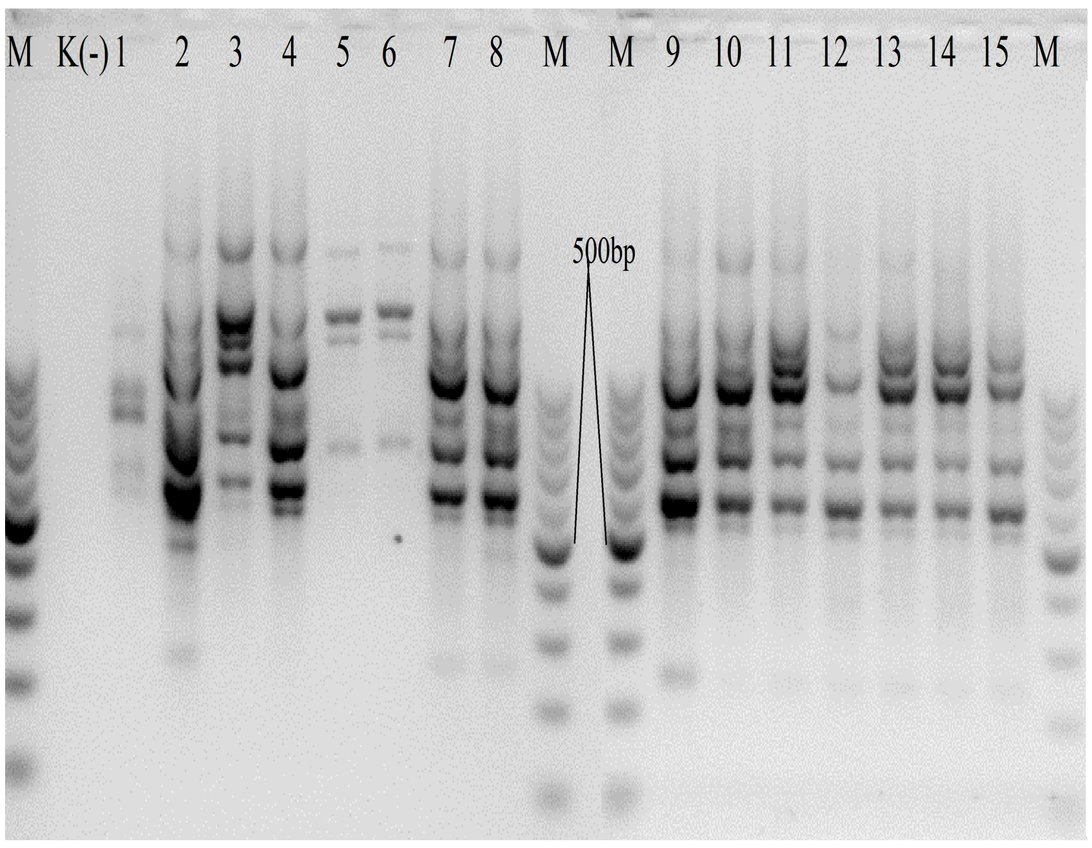

Fig. 2 Electrophoretic patterns of PCR products with $(GACA)_4$ primer:
M: 100–1000 bp Ladder (A&A Biotechnology), K(-):negative control with water,
Lanes 1-15: examined samples from culture of *Trichophyton mentagrophytes*

Results: From all collected and analyzed by conventional 'gold standard' diagnostics (direct microscopy and culture) specimens 20% yielded positive results for dermatophytes. The isolates obtained in culture and identified conventionally on the basis of their morphology included only representatives of the *Trichophyton mentagrophytes* complex strains. Pan-dermatophyte PCR assay confirmed correct identification of strains as dermatophytes. Sequenced products showed 100% agreement between strains and 99% identity with the sequences of *Arthroderma benhamiae* (the teleomorph of *Trichophyton mentagrophytes*) in GenBank. $(GACA)_4$ primer was able to differentiate all strains - The obtained fingerprints yielded up to 9 bands, ranging from approximately 250 to over 1,000 bp in length.

Conclusion:

- The method used in this investigation are proper to confirm the presence of *T. mentagrophytes* -specific DNA in pure cultures.
- $(GACA)_4$- based PCR has utility for differentiation of *T. mentagrophytes* variants as a simple, rapid, and reproducible technique.
- Foxes may be also carriers of dermatophytes.