SUMMARY

Dermatophytes are filamentous fungi naturally occurring in the soil. The fungi have a capacity to break down keratin, and are characterized by close genetic and physiological affinity, as well as an ability to cause infections. The group comprises species of genera *Epidermophyton*, *Microsporum* and *Trichophyton*, which are pathogenic for both people and animals. The infections may be located in the skin, nails and hair.

As a result of excessive growth, the host's tissues are affected by superficial fungal infection called dermatomycosis. Due to both the high incidence of dermatophytes in the human environment and the current lifestyles, the number of infections caused by dermatophytes is constantly growing. Significant from the point of view of epidemiology, symptom-free carriers, such as cats, can be a major source contributing to the transmission of infections to humans and other animals.

For years dermatophytes have posed serious problems in the area of both mycological diagnostics and therapy. Accurate and prompt identification of dermatophytes is necessary for clinical, epidemiological and preventive reasons.

It is not only the growing number of infections but also the species-related changes in the etiological factors of dermatomycoses, which have been observed over the years, that make it necessary for seek new reliable methods for fast, cost-effective and dependable identification of dermatophyte species.

The routine laboratory-based diagnostics of dermatophytes comprises a number of stages, the most important of which focuses on the species identification. Dermatophyte species identification is based on phenotype testing, yet the number of available tools is small in comparison to other groups of pathogenic fungi.

Large variability of the characteristics used in dermatophyte species identification leads to numerous errors. Moreover, the long time of growth due to dermatophyte biology additionally extends the duration of the diagnostic process. The fungi cultivated on artificial substrates tend to demonstrate variability in both the features of the colony morphology and physiological qualities, depending on the conditions of cultivation, in particular if the cultures are maintained for longer period of time. In laboratory cultures dermatophytes may also lose the capacity for sporulation, while spores are the key diagnostic factor enabling their phenotypic identification. Such changes may be induced by serial passage of the strain, which is frequently necessary in routine laboratory practices. The above phenomena are among the main causes of errors made in the process of dermatophyte identification performed using

routine diagnostic techniques. The findings of the doctoral research show that phenotype methods may be ineffective in identifying species, particularly if the species belong to one genus.

The generally adopted standards for diagnostic medical microbiology include the requirement for identifying pathogenic organisms at the level of species. The knowledge of the species which is the etiological factor of the infection is of key importance for the final therapeutic effect and for investigating epidemiology of infections caused by dermatophytes in a given area.

Contemporary medical laboratory-based diagnostics faces the necessity to replace the imperfect routine mycological techniques with fast and sensitive molecular diagnostic methods.

Numerous research centres throughout the world and in Poland focus on designing a cost-effective diagnostic test, based on a simple procedure, which will be commonly used for identifying dermatophytes in laboratories specializing in this group of fungi. Hence, the purpose of this dissertation was to examine the use of molecular methods in clinical diagnostics of dermatophytes enabling explicit identification, intraspecific differentiation and comparison of phylogenetic relationship of isolated strains occurring in the Podkarpackie Province.

The first stage of the study was designed to verify phenotypic identification of the collected strains by means of a molecular method examining the species-specific restriction fragment length polymorphism (PCR-RFLP). The acquired findings showed that phenotypic diagnostic methods may be unreliable, and this confirmed the need for exploring new effective techniques of dermatophyte identification.

Additionally, the degree of intraspecies differentiation in part of the acquired dermatophyte collection was determined using PCR-MP method based on profiles of DNA fragments with varied melting temperatures (PCR melting profile). The obtained findings demonstrated that infections induced by dermatophytes in the Podkarpackie Province were caused by 2 molecular genotypes/clones of the species *T. interdigitale.*

The second stage of the study involved the development of species-specific probes, based on the technique of fluorescent genomic *in situ* hybridisation, for identification of 3 dermatophyte species most frequently inducing infections, i.e. *M. canis, T. rubrum* and *T. interdigitale*. The findings confirmed that the developed GISH probes were characterized by high sensitivity and species-specificity. The developed species-specific probes made it possible to confirm the species of the clinical strains for which different

patterns of restriction were obtained with PCR-RFLP method. Thanks to RFLP, it was confirmed that *in situ* hybridisation may be a new and valuable diagnostic tool.

The study additionally examined the activity of 6 selected compounds of plant origin related to the reference strains of dermatophytes. The acquired findings showed varied activity of these compounds with respect to the relevant species, depending on the type and concentration of the applied substances. The greatest activity of flavonoids was observed in the case of *T. rubrum* and *M. canis*. These findings suggest potential usefulness of nutracetics in therapies.

The present findings show that molecular biology techniques make it possible to precisely identify the species of these pathogenic fungi, and to accurately determine the relation between the specific species and clones passed round in human and animal populations. Molecular and genetic methods should aid, supplement and correct the results obtained using phenotypic methods of microbiological diagnostics.