The prevalence of *Malassezia* species on the skin of HIV-positive patients

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Objective

Over the last decade there has been a growing number of reports on the prevalence of *Malassezia* species on the skin of healthy individuals and patients with *Malassezia*-associated diseases. However, only scanty information have been provided on the occurrence of *Malassezia* species specifically in patients with a severely impaired immunological status. The purpose of this study was to investigate the species composition of *Malassezia* microflora on the skin of patients positive for human immunodeficiency virus (HIV) and to describe the genetic diversity of isolated *Malassezia* strains.

Methods

The study included 29 HIV-positive subjects (26 males, 3 females, aged 23-54 years; median age: 37 years), in- and out-patients of the Chair of Gastroenterology, Hepatology and Infectious Diseases, Medical College, Jagiellonian University, Cracow. From each patient, samples from four different body sites (head, face, chest, and back) were collected by a swab method. The yeasts were cultured on modified Dixon's agar medium, and the suspected colonies of *Malassezia* were subcultured, and subjected to species identification by using both phenotype-based and molecular methods. Whereas conventional differentiation involved an array of biochemical tests, molecular speciation was done with PCR-sequencing of the internal transcribed spacer (ITS) 1/2 regions within the rDNA operon (Fig.1.). The sequence data were analysed using ClustalX, and the phylogenetic tree was constructed with MEGA6 software by using the Maximum Likelihood method and Kimura 2-parameter model.

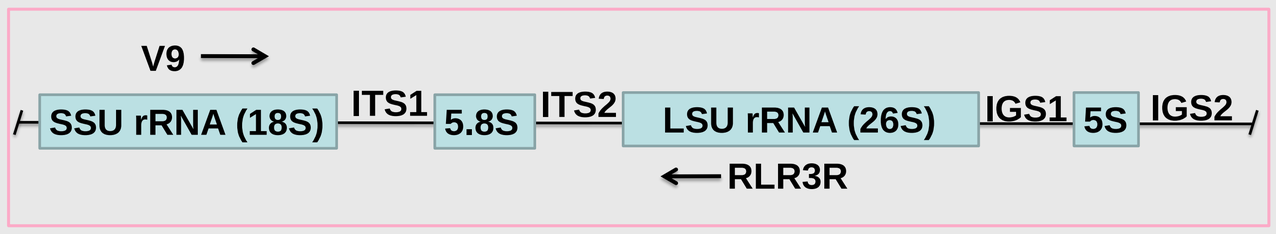

FIGURE 1. Schematic representation of the rDNA operon in *Malassezia* yeasts. Primers used for PCR amplification and sequencing are indicated as black arrows. LSU, large subunit (rRNA); SSU, small subunit (rRNA); IGS, intergenic spacer region; ITS, internal transcribed spacer.

Results

A total of 57 *Malassezia* cultures were obtained from clinical samples, with back being the commonest site of isolation (21 cultures; 36.9% of all *Malassezia* cultures), followed by chest (20; 35.1%), face (8; 14%), and head (8; 14%). The overall positive culture rate of the *Malassezia* yeasts was 49.1% (57 positive samples out of 116 samples tested). *Malassezia sympodialis* was recovered with the highest frequency (i.e. from 27 or 47.3% of the samples), followed by *M. globosa* (17; 29.8%), *M. furfur* (10; 17.5%), *M. dermatis*, *M. restricta*, and *M. slooffiae*, with the latter three being cultured from single samples only. No correlation was found between the *Malassezia* species identified and anatomical site or skin condition. The phenotypic and molecular methods used for species identification gave concordant results for 54 (94.7%) of the strains cultured. Three strains classified as *M. sympodialis* (1) and *M. furfur* (2) by phenotypic methods, were designated as *M. obtusa*, *M. sympodialis* and *M. globosa,* respectively by sequece analysis.
The rDNA sequences demonstrated a low degree of intra-species diversity.

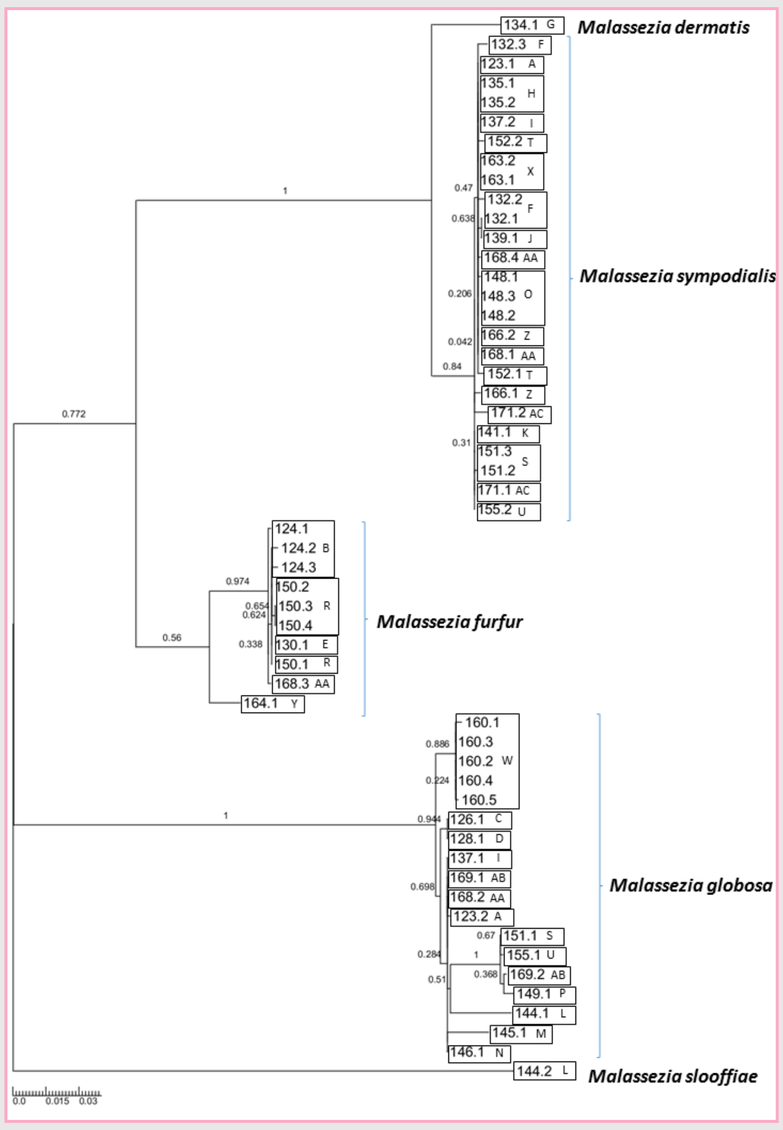

FIGURE 2. Phylogenetic tree constructed using the rDNA sequences of *Malassezia* strains isolated from HIV-positive subjects.
Numbers and letters in brackets represent different strains collected from patients and patients themselves, respectively. Bootstrap values (with 500 resamplings) are represented at internal nodes for values >0.2.
(Note that sequences of two strains – *M. sympodialis* (159) and *M. restricta* (153) were not included in the dendrogram, as they were too short, compared with the sequences from all the remaining strains).

Conclusions

The species composition of *Malassezia* microbiota on the skin of HIV-positive subjects seems not to differ from that observed among individuals of either negative or unknown HIV status. Despite the high concordance of the two identification approaches, PCR-sequencing is a preferred method, as its results are more reliable and more easily and rapidly produced.

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The study was approved by the Ethical Committee of the Jagiellonian University (KBET/33/B/2011).