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Molecular identification of
Malassezia species isolated from animals

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SUMMARY

Malassezia is one of the most important yeast genera, which causes Malasseziosis in different animals. It can be transmitted to humans by contact with pets. The outcome of this work dealt with the methods of phenotypic and genotypic identification of Malassezia isolated from dogs, cats, horses, and buffaloes.

By direct microscopy using 10% KOH, fungal elements related to *Malassezia* spp. were observed in 32.05% (25/78) of skin scrapings from diseased (31.2%) and apparently healthy animals (33.3%). The identified *Malassezia* spp. were highly observed in dogs (47.82%, 11/23), followed by cats (33.3%, 5/15), horses (25%, 6/24) and buffaloes (18.75%, 3/16).

Out of 160 samples (82 ear swabs and 78 skin scrapings) from apparently healthy and diseased animals, 49 isolates; 24 from ear swabs (29.27%) and 25 from skin scrapings (32.05%) yielded positive growth on mycobiotic agar medium were suspected to be Malassezia and subjected to phenotypic and genotypic identification methods.

Phenotypic methods for identification of the recovered isolates includes macro and micromorphological characters as well as growth on mycobiotic agar medium at different temperatures, tween assimilation, esculin hydrolysis, tryptophan utilization, and catalase tests were used.

On mycobiotic agar medium with olive oil, lipid dependent species (*M. globosa*) showed creamy and rough colonies, whereas non-lipid dependent species (*M. pachydermatis*) on media without oil revealed raised, creamy and smooth colonies.

Concerning microscopy after Gram`s stain, *M. pachydermatis* were cylindrical to oval yeast cells with broad base buds, grew at 31, 37 and

40˚C on mycobiotic agar medium, assimilated all tweens and they all were negative for both tryptophan utilization and esculin hydrolysis tests. The only *M. globosa* isolate yielded spherical yeast cell with narrow based buds, failed to grow on mycobiotic agar medium at 40˚C, gave positive results for catalase test, did not assimilate all tweens and it was negative for both tryptophan utilization and esculin hydrolysis tests.

Out of 49 recovered *Malassezia* spp., 48 were identified as *M. pachydermatis* and only one *M. globosa.*

Twenty-one representative Malassezia isolates formally identified according to their phenotypic characters (20 were identified as *M. pachydermatis* and one *M. globosa*) were subjected to PCR-RFLP assay. PCR amplification of 26S rDNA gene from all tested *Malassezia* spp. revealed a single PCR product of the expected size at 580 bp.

Digestion of the amplicons with *Hha1* restriction enzyme revealed four restriction patterns specific for *M. pachydermatis* (n=17), *M. furfur* (n=1), *M. globosa* (n=2) and *M. restricta* (n=1).

Plainly, 17 out of 21 *Malassezia* spp. isolates were correctly identified by PCR-RFLP assay. Three isolates were correctly identified as *M. globosa, M. furfur* and *M. restricta.*

It revealed that 17 *M. pachydermatis* were isolated from all animals' host; dogs (7), cats (5), horses (3) and buffalos (2). Moreover, 2 *M. globosa* were isolated from horse and buffalo (one each). Finally, one *M. furfur* and one *M. restricta* were isolated from buffalo and dog respectively.

PCR-RFLP showed 2 bands specific for *M. globosa* (129 and 455 bp), 3 bands for *M. pachydermatis* (97, 221 and 250 bp), one band for *M. restricta* (580 bp) and 3 bands for *M. furfur* (107, 113 and 250 bp).

Subsequently, five representative isolates identified by phenotypic and PCR-RFLP assay were subjected to DNA sequencing of 26S rDNA region.

The GenBank accession numbers of nucleotide sequences were MK351279 for *M. furfur* that was isolated from skin scrapings of diseased buffalo, MK351310 and MK351317 for *M. globosa,* from skin scrapings of diseased horse and apparently healthy buffalo, respectively. MK351319 for *M. pachydermatis* from ear swab of apparently healthy cat and MK351315 for *M. restricta* from skin scrapings of diseased dog.

Concordance between PCR-RFLP and DNA sequencing was 100%. A Phylogenetic tree built from the obtained sequences showed different clusters for each species, indicating variation in their sequences. The identified sequences for all species were clustered with those previously deposited at GenBank for the same species.

Phenotypic methods identified only *M. pachydermatis* and *M. globosa*, while molecular method successfully identified *M. pachydermatis, M. globosa, M. restricta* and *M. furfur*. There are significant differences between phenotypic and PCR results *(P.* value = 0 .019). The relative sensitivity and accuracy of PCR-RFLP assay were 100% and 86%, respectively.

Although the phenotypic methods could identify some *Malassezia* spp., the PCR-RFLP assay using *Hha1* restriction enzyme and DNA sequencing of 26S rDNA region are complementary and mandatory for identification of *Malassezia* spp. isolated from animals.