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YEASTS OF THE *MALASSEZIA* GENUS – RECENT FINDINGS

Malinovská, Z., Čonková, E., Váczi, P.
Department of Pharmacology and Toxicology
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

zuzana.malinovska@uvlf.sk

ABSTRACT

The genus *Malassezia* is a medically important genus of yeasts that can colonize the skin of humans and other warm-blooded animals. The genus currently comprises 18 species of which four new species were identified recently. The most widely known species, *M. pachydermatis*, occurs in animals but was detected also in humans, namely at life endangering septicaemias and in prematurely born children. Proliferation of *Malassezia* occurs most frequently as a result of disturbances in the normal homeostasis of host immunity on the one hand and virulence of these yeasts on the other hand. The successful management of the disease depends on the therapeutic control of overgrowth of the yeasts and any concurrent bacterial infection by local or systemic antimicrobial treatment, as well as, on identification and potential correction of the predisposing factors.

Key words: cultivation; *Malassezia* spp.; occurrence; virulence

INTRODUCTION

Yeast infections are a relatively common problem in a small animal veterinary practice. In dogs and cats the genera *Malassezia* and *Candida* are the most frequently encountered opportunistic organisms. They can be a part of the normal microbiota of skin and mucous membranes of these animals, not acting as pathogens, but under certain conditions, particularly those with weakened local and total immunity of an individual, they may become pathogenic and cause mycosis, either as a primary disease or as a secondary infection [5].

Yeasts of the genus *Candida* are associated more likely with mucosal epithelium and in dogs they are the main aetiological agents of infections of the oral cavity, the gastrointestinal and the urogenital tracts. In comparison with *Candida*, *Malassezia* yeasts are more associated with skin and are the most frequent cause of otitis and dermatitis. The prevalence of inflammation of the external auditory canal in dogs in veterinary ambulance may reach 10 % and as many as 70 % of such cases may be related to the yeast of the *Malassezia* genus [18, 34].

Species of the genus *Malassezia*

Only three *Malassezia* species were known until 1990. With the development of molecular biological methods, new species were gradually identified. Currently, the following 18 species have been identified: *M. pachydermatis*, *M. furfur*, *M. sympodialis*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. dermatis*, *M. japonica*, *M. nana*, *M. yamatoensis*, *M. caprae*, *M. equina*, *M. cuniculi*,

Table 1. Occurrence of individual species of *Malassezia* in animals and man [30, 44]

Malassezia spp.	Principal host/other hosts	Year of 1st identification
M. pachydermatis	Dog, cat/ruminants, pig, cow	1925 (Dodge) [14]
M. furfur	Man/cow, pig	1989 (Baillon) [3]
M. sympodialis	Man/horse, pig, ruminants	1990 (Simmons, Guého) [39]
M. globosa	Man/cow	1996 (Guého et al.) [21]
M. obtusa	Man	1996 (Guého et al.) [21]
M. restricta	Man	1996 (Guého et al.) [21]
M. slooffiae	Man, pig/cat, ruminants	1996 (Guého et al.) [21]
M. dermatis	Man	2002 (Sugita et al.) [43]
M. japonica	Man	2003 (Sugita et al.) [42]
M. nana	Cat, cow	2003 (Hirai et al.) [23]
M. yamatoensis	Man	2004 (Sugita et al.) [41]
M. caprae	Goat/horse	2007 (Cabañes et al.) [7]
M. equina	Horse/cow	2007 (Cabañes et al.) [7]
M. cuniculi	Rabbit	2011 (Cabañes et al.) [8]
M. brasiliensis	Parrot	2016 (Cabañes et al.) [9]
M. psittaci	Parrot	2016 (Cabañes et al.) [9]
M. arunalokei	Man	2016 (Honnavar et al.) [24]
M. vespertilionis	Bat	2018 (Lorch et al.) [30]

M. brasiliensis, *M. psittaci*, *M. arunalokei* and *M. vespertilionis* (Table 1) [30, 44].

Some species from the genus *Malassezia* (particularly *M. pachydermatis*) have a broad spectrum of hosts while other are more specific and occur only in one animal species or in a group of related animals and in man. The use of genotype identification methods, e. g. PCR-RFLP, RAPD, sequencing and other, is inevitable for accurate species diagnosis of *Malassezia*. Phenotype methods developed for distinguishing of species of the genus *Malassezia* on the basis of characterization of cultural and biochemical properties differ which makes their definite identification more difficult. Moreover, individual *Malassezia* species include a wide range of genotypes with different phenotype properties even within particular species [11, 12, 16, 20, 26, 46].

The presence of *Malassezia* in pet animals, such as dogs or cats are more common than in farm animals. *Malassezia* were isolated from both healthy animals and those suffering from otitis and dermatitis. The yeast isolated most frequently from dogs is *M. pachydermatis*. *M. furfur* was identified in dogs, but very rarely. The *M. nana* and *M. slooffiae* species were diagnosed in cats. In comparison with dogs, *M. pachydermatis* occurs in cats far less than in dogs [2, 31, 32].

Information about the occurrence of *Malassezia* in farm animals is very scarce. The clinical role of *Malassezia* in farm animals is not clear, also with regard to the fact that the presence of *Malassezia* in these animals have been investigated very little. Their occurrence in farm animals was confirmed more frequently in healthy individuals and only sporadically in animals suffering from dermatitis or otitis; maybe due to the small number of studies concerned with the presence of *Malassezia* in sick animals. In cattle, the following species were identified by use of the genotyping methods: *M. furfur*, *M. sympodialis*, *M. nana*, *M. slooffiae*, *M. pachydermatis* and *M. equina* [7]. Of *Malassezia* species occurring in small ruminants there were identified as *M. caprae*, *M. slooffiae* a *M. sympodialis* [6, 7, 45]. The most typical *Malassezia* species

identified in horses is *M. equina* (most frequently isolated from skin around the anus). From healthy skin of horses there were sporadically isolated and genotypically determined also as *M. caprae*, *M. sympodialis* and *M. slooffiae* [6, 7, 47]. In pigs there were confirmed species, such as *M. furfur*, *M. sympodialis*, *M. slooffiae* and also *M. pachydermatis* [19, 33]. *M. cuniculi* was isolated from rabbits in New Zealand [8]. The mentioned studies originate from countries such as Spain, Italy, United Kingdom, Brazil and California with typical subtropical climates characterised by higher humidity and lower temperature differences and differing from that in the Czech and Slovak republics. It was observed that the occurrence of *Malassezia* in farm animals in Slovakia is very scarce (even none). The testing of 223 cattle detected *M. pachydermatis* only in two animals. Another species, *M. sympodialis*, was identified in one animal from 77 tested pigs. In the Slovak territory, no *Malassezia* were isolated from horses, small ruminants or rabbits [38].

New species of the genus *Malassezia*

The increasing availability of genomic and molecular tools and methods played a key role in the determination of new species of the genus *Malassezia*. Sequencing of the genome of various field isolates of *Malassezia* allowed scientists to uncover recently as many as four new species.

In 2016 two new species, *M. brasiliensis* and *M. psittaci*, were uncovered in five pet parrots in Brazil after sequencing which showed that they did not correspond to sequences of that time known species. *M. brasiliensis* and *M. psittaci* were isolated from lesions on the beak and oropharynx of the parrots and their properties were similar to those of other species of the genus *Malassezia* [9].

In 2016, *M. arunalokei* was recognized as a new species. It was isolated from scalp skin of a human patient with seborrheic dermatitis in Chandigarh in India in December 2012 and was confirmed as a new species four years later. It was identified in patients with moderate to medium serious seborrheic dermatitis but also in healthy individuals. Recently, it was determined that it occurs more frequently on the forehead and cheeks. It is interesting that phylogenetically *M. arunalokei* is very close to the species *M. restricta*, but it was determined that it deflected from this species 7.1 million years ago [13, 24].

In 2018, new species, *M. vespertilionis*, was isolated from bats in the eastern and western states of the USA. The isolates were obtained by swabbing the skin of the wings of hibernating bats in the subfamily Myotinae. The cultivation properties of this newly discovered species were distinct from the other *Malassezia*. The typical temperature of growth of *Malassezia* spp. is 32 °C (some species are able to grow even at 40 °C) but this new species grows best at 24 °C. It is able to grow over a broad temperature range – it can be cultured already at 7 °C up to the maximum of 40 °C. This thermal growth range confirms the adaptation by the yeast to survive on the skin of bats during both the host's hibernation and active seasons. The culturing period of *M. vespertilionis* can require as many as 50 days while with other species it usually lasts 2–14 days [30].

Lipid dependence of *M. pachydermatis*

M. pachydermatis was long considered a lipophilic but not lipid-dependent species, capable of growth without addition of lipids, namely on Sabouraud dextrose agar. This specific property was frequently used as a rapid diagnostic method to distinguish it from other lipid-dependent species. Recent genome sequencing determined that *M. pachydermatis* lacks the fatty acid synthase gene present in other species of this genus [48]; but is able to use for its growth the lipid fractions of the peptone component of the Sabouraud dextrose agar. The dependence of *M. pachydermatis* on lipids was confirmed by its failure to grow during culturing on a special lipid-free medium [36].

Complex evaluation of clinical cases

Persistent or recurrent *Malassezia* dermatitis or otitis in dogs is usually associated with insufficient identification and elimination of predisposing or unceasing factors. Clinical disease associated with the overgrowth of *Malassezia* frequently reflects proliferation of yeasts due to the disturbances of normal homeostatic balance of a host's immunity on the one hand and virulence of the yeasts on the other hand. Dog breeds with increased risk of *Malassezia* dermatitis include: West Highland White Terrier, English Setter, Shi Tzu, Basset Hound, American Cocker Spaniel, Boxer, Dachshund, Poodle and Australian Silky Terrier. Predisposed are also cat breeds Devon Rex and Sphynx [2]. The localization on the body of animals is derived from the lipophilic character of these yeasts. The most frequently inhabited sites are: the auditory canal, face, skin folds, interdigital space, axillary and inguinal region and anal region [22].

The successful management of *Malassezia* diseases frequently depends on the treatment of yeast overgrowth and concurrent bacterial infection by local or systemic antibiotics, as well as, identification and potential correction of predisposing factors.

The proliferation of yeasts is linked to factors such as: hypersensitivity (atopy, skin food allergies, allergy to flea bite and contact allergy), anatomic defects (skin folds or serous auditory canals), cornification disorders, ectoparasite infections, bacterial pyodermy, and endocrine disorders (hyperadrenocorticism, hypothyroidism, *diabetes mellitus*, in cats also viral leukaemia and viral leukopenia). Long-term corticoid or antibiotic treatment can also result in an increase of yeast populations. In addition, lesions can develop due to hypersensitive response to yeast allergens [5].

Virulence factors may play an important role in the pathogenicity of *Malassezia*. These factors include: enzymes esterase, lipase, acidic phosphatase, lipoxygenase, protease, phospholipase and proenzyme zymogen of the cellular wall of yeasts that can activate the complement system, initiate damage to integrity of keranocytes and result in epidermal spongiosis, inflammation and pruritus. Relevant studies have demonstrated that the expression of phospholipase in *M. pachydermatis* is modified by endogenous opioid peptide β-endorphin, which is facilitated by receptors present in the cell wall of *M. pachydermatis*. It was assumed that under the action of suitable agonists (β-endorphin) or antagonists (naloxone), these receptors affect the development of commensal or pathogenic phenotype in this species [10]. The pathogenic role of *Malassezia* may be related to the changes in chemical or immunological mechanisms of the skin that may modify the composition of the cell wall of *Malassezia*. The composition of the cell wall of the yeast *M. restricta* confirms that this is a specific type of yeast in the fungal kingdom. The cell wall of this species contains an average of 5 % chitin, 20 % chitosan, 5 % β-(1,3)-glucan and 70 % β-(1,6)-glucan that form a large alkali-insoluble complex [40].

Resistance

When dealing with a clinical case, the attending veterinarian does not need inevitably to know the species identity of *Malassezia* that caused the disease. The diagnosis on the level of yeast genus, i. e., genus *Malassezia* spp., suffices for the treatment. More important is the determination of antifungal susceptibility of the relevant clinical isolate (the agent). The cases of reduced susceptibility of *Malassezia* to commonly used antimycotics detected under field and laboratory conditions indicate the necessity of control and alertness with antimycotic treatment. In cases of treatment of recurrent or persistent mycosis, repeated testing of susceptibility of relevant isolates is recommended. It was observed that "resistant" cases were in the past 12 months treated on average by 4.4 cycles of antimycotics while dogs with "sensitive" isolates received on average only 0.8 cycles. This observation supported the view that this involved acquired resistance of yeast isolates. The latest research confirmed that increasing frequency of azole resistance in field strains, that can cause problems with the treatment, as azoles are among the most frequently used antimycotics and generally are considered as the most effective drugs for the therapy of *Malassezia* mycosis. Concrete genes, e. g. ERG11, ATM1, PDR10 and others, have been regarded as responsible for resistance of *Malassezia* [27, 29, 35, 37].

The resistance of *Malassezia* is closely related to formation of biofilms. The formation of biofilm is an important factor of virulence of yeasts of the genus *Malassezia* and a serious issue concerning future antimycotic therapy. Biofilm cells are protected against the action of antimycotics in several ways. The biofilm environment allows the cells to multiply and protects them against the immune system of the host and the effect of antifungal compounds. Investigations confirmed the ability of *M. pachydermatis* to form biofilms and much lower susceptibility of biofilms of all tested strains to ketoconazole and itraconazole in comparison to susceptibility of their planktonic forms. It was also observed that the cells persisting in biofilms exhibited increased expression of genes responsible for their resistance [25].

New therapeutic possibilities

The increasing antimycotic resistance of *Malassezia* has encouraged the search for new ways of therapy. Recently the role of plant essential oils as antimicrobial compounds became the subject of intensive research because of their documented action on fungal, viral and bacterial pathogens [1, 17]. Essential oils are mixtures of volatile oils obtained from plants, soluble in alcohol and aether but insoluble in water. Plant essential oils contain a broad range of chemical compounds capable of acting

by multiple mechanisms on various components of microbial cells. During the past five years, scientific studies described properties of a large number of tested essential oils obtained from plants in various genera: *Thymus* (thyme), *Artemisia* (wormwood), *Melaleuca* (tea tree), *Cinnamonum* (cinnamon), *Ocimum* (basil), *Rosmarinus* (rosemary), *Origanum* (oregano), *Syzygium* (clove), *Foeniculum* (fennel), *Trachyspermum* (ajwain), *Myrtus* (myrtle), and others. A number of studies confirmed that plant essential oils are promising effective components in fighting the *Malassezia* yeasts [4, 15, 17, 28].

CONCLUSIONS

Lipophilic *Malassezia* spp. are commensal fungi of the mammalian skin, responsible for dermatitis and otitis in dogs. It has been recognised that they may be the cause of primary mycosis, but as well as responsible for concurrent infection at bacterial diseases of skin and ears. They represent a risk factor at some dermatitis types, e. g., atopic or seborrheic. Proliferation of *Malassezia* occurs as a result of weakened immunity of an individual and is supported by virulence factor of *Malassezia* in the form of various enzymes and proenzymes. *Malassezia* mycoses are frequently characterised by relapses and successfulness of their treatment requires a complex approach.

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