Antifungal effect of Australian tea tree oil on *Malassezia pachydermatis* isolated from canines suffering from cutaneous skin disease

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Summary

The lipophilic yeast *Malassezia pachydermatis* is part of the normal skin flora of most warm-blooded organisms. In a number of surveys it could be demonstrated that this yeast species might be involved in different skin diseases like seborrhoic dermatitis, especially in dogs and cats. In order to look for an alternative therapeutic agent to the commonly used antimycotic and antiseptic synthetic substances the *in vitro* activity of Australian tea tree oil, the essential oil of *Melaleuca alternifolia,* against several strains of *Malassezia pachydermatis* was examined. All tested strains showed remarkably high susceptibility to tea tree oil. With these results the excellent antibacterial activity of tea tree oil is extended to a new group of fungal pathogens colonizing mainly mammals' skin. During the last ten years there was an increasing popularity of tea tree oil containing human health care products. The presented data open up new horizons for this essential oil as a promising alternative agent for topical use in veterinary medicine as well.

Key words: Malassezia pachydermatis – dog – tea tree oil – antifungal activity – dermatitis

Zusammenfassung

Der Hefepilz *Malassezia pachydermatis* ist bei den meisten warmblütigen Organismen Teil der normalen Hautflora. In einer Reihe von Übersichtsarbeiten wurde darauf hingewiesen, dass dieser Hefepilz vor allem bei Katzen und Hunden an der Ausbildung verschiedener Hauterkrankungen, wie z.B. der seborrhoischen Dermatitis, beteiligt ist. Als Alternative zu synthetischen Antimykotika und Antiseptika, die üblicherweise zur Behandlung von leichten oberflächlichen infektiösen Hauterkrankungen eingesetzt werden, wurde *in vitro* die antimykotische Aktivität von Teebaumöl, bei dem es sich um das ätherische Öl von *Melaleuca alternifolia* handelt, gegen verschiedene Stämme von *Malassezia pachydermatis* untersucht. Alle getesteten Stämme zeigten eine bemerkenswert hohe Empfindlichkeit gegen Teebaumöl. Durch dieses Ergebnis konnte die ausgezeichnete antimikrobielle Wirkung von Teebaumöl bestätigt und um einen weiteren pathogenen Keim, der vor allem die Haut von warmblütigen Organismen besiedelt, erweitert werden. In den vergangenen zehn Jahren erlangte Teebaumöl grosse Popularität als Bestandteil von Gesundheitsprodukten im Humanbereich. Die vorgelegten Ergebnisse eröffnen für Teebaumöl auch in der Veterinärmedizin Anwendungsmöglichkeiten, z.B. als vielversprechende Altenative zu synthetischen Mykotika bei äusserlicher Anwendung.

Schlüsselwörter: Malassezia pachydermatis – Hund – Teebaumöl – antimykotische Wirkung – Dermatitis

Introduction

Malassezia pachydermatis is a lipophilic non-lipid dependent basidiomycetous yeast with short oval to ellipsoidal cell shapes and a typical unipolar budding reproduction procedure (Fig.1). The fungus is part of the normal cuteanous microflora of a variety of domestic and wild warm-blooded animals, and seldom of human being. In 1925, *Malassezia pachydermatis* was first described by

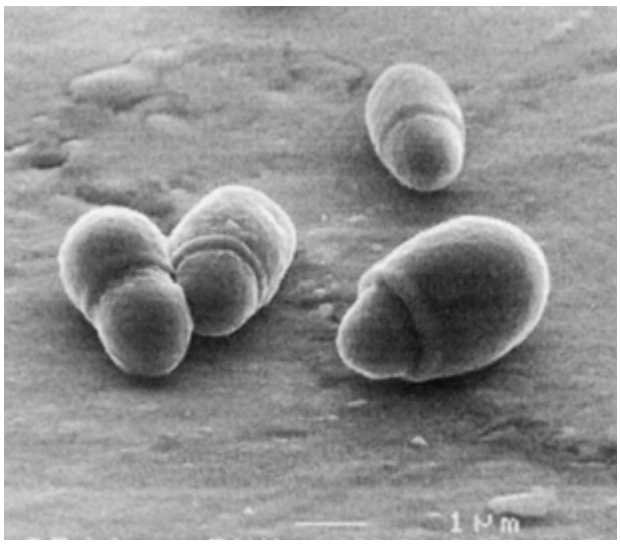

Fig. 1: Scanning electron micrograph of Malassezia pachydermatis. Unipolar budding yeast cells with prominent collarettes are easily recognized (Guillot and Bond, 1999).

Weidman as *Pityrosporum pachydermatis* (Weidman, 1925). He isolated this microorganism in scales from an Indian rhinoceros *(Rhinoceros unicornis)* with exfoliative dermatitis. Because of its resemblances to *Pityrosporum ovale,* the purported agent of human dandruff, he classified this yeast to the genus *Pityrosporum*. In the following five decades several strains of *Malassezia* and *Pityrosporum* respectively were found in dogs and described with different names by their investigators. Until 1970, Sloof summarized all strains of *Pityrosporum* growing on media without lipid supplementation to a single species named *Pityrosporum pachydermatis* (Sloof, 1970). There was an agreement about the use of *"Malassezia"* and *"Pityrosporum"* as synonymous terms in 1984 and the anteriority for the generic *"Malassezia"* was generally accepted (Yarrow, Ahearn, 1984). Based on genomic studies *Malassezia* yeasts could be distributed among six lipid-dependent distinct taxa *(M. furfur, M. sympodialis, M. globosa, M. obtusa, M. restricta and M. sloofiae)* and one non-lipid-dependent strain *Malassezia pachydermatis* (Guého et al., 1996; Guillot et al., 1995).

Human skin is usually colonized by lipid-dependent *Malassezia* species, especially *Malassezia furfur.* Therefore most of their skin infections due to these fungi were induced by *Malassezia furfur* and implicate superficial infections of skin and associated structures. Nevertheless *Malassszia furfur* as well as *Malassezia pachydermatis* were recently found to be the causing agents for a number of invasive human infections. Especially an intravascular catheter-acquired sepsis in neonates during hospitalisation in intensive care units was associated with these microorganisms (Marcon and Powell, 1992). In contrast to that most of the wild and domestic carnivores' skin, e.g. of dogs, cats, bears, ferrets and foxes, *Malassezia pachydermatis* could be recovered. In a number of studies it could be clearly demonstrated, that there is a high incidence rate of skin disease in pets. Particularly in dogs and cats *Malassezia pachydermatis* played an important role in the pathogenesis of otitis externa and seborrhoic dermatitis (Guillot and Bond, 1999). Gustafson (1955) induced otitis externa by applying *Malassezia pachydermatis* in the external ear canal of his experimental dogs, whereas a *Pityrosporum ovale* strain of human origin had no effect. Although there is the current opinion that *Malassezia pachydermatis* does not usually act as a primary but rather as an opportunistic secondary pathogen under poorly defined circumstances. It is suggested that whenever alternations in the skin surface micro climate and host defence occur, e.g. induced by trauma, foreign bodies, ectoparasites, bacteria and allergens usually commensal micoorganisms may become pathogenic. Other investigators observed that certain breeds of dogs seemed to be predisposed for ear infections if they have long and bent ears and abundant hair growth in the ear canal (Kuttin and Glas, 1985). Additionally, a high colonization rate of the yeast was found on parts of the body with poor air circulation on hairy skin, e.g. interdigital areas, areas at the lower lip and of the anus, rectum, anal sacs and vagina. In most of the cases the diagnosis of these infected skin lesions was characterized by pain, unrest, pruritus, discharge from the ear with a sometimes stinking smell, erythema and a local loss of hair (Fig. 2). In order to present a proper treatment of the sick animals it was necessary to make direct smears and cultures to get information about the germs involved in the inflammation. In addition to a high amount of *Malassezia* positive isolates a concurrent colonization and infection by coagulase-positive staphylococci such like *Staphylococcus aureus* and *Staphylococ-*

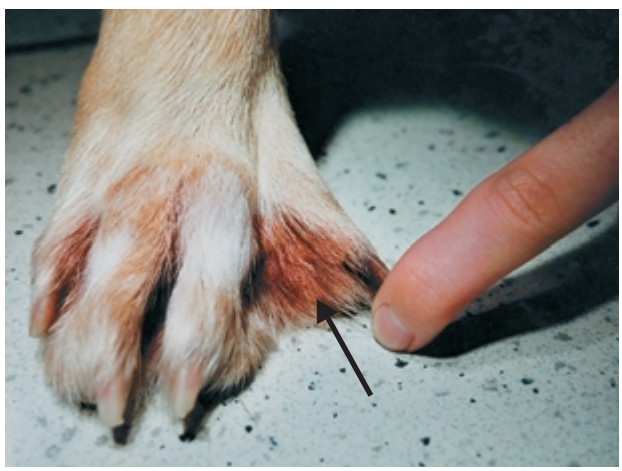

Fig. 2: Clinical manifestation of Malassezia pachydermatis associated pyoderma at the interdigital area of dog's paw (Fig. Bogar AG, Zürich).

cus intermedius has been frequently demonstrated. The best therapeutic response was therefore achieved by a topical therapy using a combination of antimycotic and antiseptic substances that removed both, *Malassezia pachydermatis* and bacteria. In clinical practice miconazole or ketoconazole shampoos combined with chlorhexedine or selenium sulphide shampoos have been commonly used (Guillot and Bond, 1999).

In our previous studies we investigated the antibacterial activity of Australian tea trea oil (TTO), the essential oil of *Melaleuca alternifolia* against a wide spectrum of gram-positive and gram-negative bacteria as well as different *Candida* species (Reichling et al., 1997; Reichling et al., 1999). Generally germs were widely susceptible to TTO in concentrations between 0.25 and 1.0% (v/v). Current research work related to its antifungal effects, mainly focused on human pathogenic fungi, especially on *Candida* yeasts and *Malassezia furfur* (Hammer et al., 1997). In order to look for an alternative agent with good antibacterial as well as antifungal properties in the treatment of *Malassezia pachydermatis* associated skin lesions in animals we determined the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of TTO against *Malassezia pachydermatis* in comparison to the antimycotic substance terbinafine-HCl *in vitro*.

Material and Methods

Essential oil and terbinafine-HCl

The Australian tea tree oil tested was a commercial product of Australia and kindly provided by Bogar AG, Switzerland. The antimycotic reference substance terbinafine-HCl was a kindly gift from Novartis AG, Germany.

GC method

The major components of the essential oil were analysed in n-hexane as solvent. GC was performed using a Hewlett Packard HP 5890 chromatograph. The GC column was a 30 m × 0.32 mm (i.d.) fused silica capillary column (DB-5) coated with 5% diphenyldimethylpolysiloxane (film thickness 0.25 μm) and with He as the carrier gas (flow rate: 1.7 ml/min.). Temperature program: The initial column temperature was 60° C for 4 min. Subsequently the temperature rate was programmed from 60° C to 240° C in two steps, first 6° C/min. up to 200° C and then 5° C/min up to 240° C. Injector temperature: 260° C; detector temperature: 320° C; injection volume: 1 μl.

GC-MS method

A gas chromatograph Hewlett Packard HP 5890 with a mass selective detection was used for GC-MS analyses. GC column: DB-5, 25 m × 0.25 mm (i.d.) fused silica capillary column coated with 5% diphenyldimethylpolysiloxane (film thickness: 0.15 μm). Temperature programm: 60° C for 4 min.; 6 ° C/min. up to 200° C; then 5° C/min. up to 240 ° C. EI ionizing voltage 70 eV.

Fungi

We examined the susceptibility of five clinical canine isolates and four certificated canine strains of *Malassezia pachydermatis* (Tab. 1) towards TTO and terbinafine-HCl, respectively. The strains were directly isolated from the skin of infected dogs and derived from the Leeds Veterinary Laboratories Ltd. Millcroft, Leeds, UK. Three of the four certificated strains were microorganisms of the National Collection of Pathogenic fungi (NCPF), Great

Table 1: Origin and identification number of the investigated Malassezia pachydermatis strains.

Fungi	Isolate number	Date of first isolation	Species
Clinical isolates			
Malassezia pachydermatis	B 7097	02.10.1999	Dog
Malassezia pachydermatis	B 7104	05.10.1999	Dog
Malassezia pachydermatis	B 7124	19.10.1999	Dog
Malassezia pachydermatis	B 7139	26.10.1999	Dog
Malassezia pachydermatis	B 7148	30.10.1999	Dog
Certificated strains			
Malassezia pachydermatis	NCPF 3595	1989	Dog
Malassezia pachydermatis	NCPF 3596	1989	Dog
Malassezia pachydermatis	NCPF 3667	1991	Dog
Malassezia pachydermatis	DSM 6172		

Britain and one strain was obtained from the DSM (Deutsche Stammsammlung), Germany.

Broth microdilution method

Determination of the MIC (minimum inhibitory concentration) and the MFC (minimum fungicidal concentration) was based on a modified broth microdilution method according to DIN regulation 58940 part 8 and appendix 1, 1997.

The investigated yeasts were cultured and tested in Sabouraud-broth (SB, Oxoid). It was shown by several investigators that the presence of Tween 80 at a final concentration of 0.5% (v/v) in the culture medium was required for optimum growth of the *Malassezia pachydermatis* strains (Bond and Lloyd, 1996; Lorenzini and de Bernadis, 1987). In addition this concentration of detergent successfully enhanced solubility of the lipophilic essential oil in the aqueous test medium, so that a separation of the lipophilic and hydrophilic parts of the test solutions was prevented. TTO and terbinafine-HCl were tested in parallel plates and treated under identical conditions. Geometric dilutions ranging from 0.03–2% (v/v) TTO and 0.01–25 µg/ml terbinafine respectively were prepared in a 96-well microtiter plate. One growth control (SB + Tween 80) and one sterility control (SB + Tween 80 + TTO) were included in each plate. The final inoculum of the test strains was adjusted to 5×10^3 cfu/ml controlled by the spiral plater counting method (Spiral Systems Cincinnati, OH, USA). Plates were incubated under normal atmospheric conditions at 37° C for 72 h. Each test was performed in duplicate and repeated twice. If there were still differences the test was done another time. The MIC is defined as the lowest concentration of the essential oil at which the microorganism being tested does not show any visible growth. The fungal growth was indicated by the presence of a white "pellet" on the bottom of the well. The MFC is defined as the lowest concentration of the essential oil at which 99.9% of the fungi have been killed. For its determination 10 µl broth was removed from each well dropped onto an SPS-agar plate and incubated at 37° C for further 72 h. After that incubation period the lowest concentration without colony growth on the agar plate was defined as MFC.

Results and Discussion

Chemical characterisation of TTO

Since the chemical composition of the Australian tea tree oil (TTO) affects fungal growth, the essential oil was characterized chemically bevor it was used in the bioassay. The major component of the essential oil were identified by their mass spectral data as well as by coinjection with authentic substances (e.g., terpinene-4-ol, 1,8-cineol). TTO consisted mainly of terpinene-4-ol (40.7%), γ-terpinen (20.4%), α-terpinene (9.1%), p-cymene (2.2%), 1,8-cineol (3.1%), terpinolene (3.3%), α-terpineol (3.1%), α-pinene (2.3%), and limonene (1.0%). The TTO was in accordance with the Australian standard (Reichling et al., 1997).

Antifungal activity of TTO

MIC and MFC results are given in Table 2. In the test battery we investigated the susceptibilty of five clinical strains as well as four certificated strains of *Malassezia pachydermatis* against TTO and terbinafine-HCl. No significant differences in the susceptibility to the tested substances were found between the clinical isolates and the certificated strains. TTO inhibited all tested strains at uniform low concentrations of 0.06–0.13% (v/v) and 560.0–1120.0

Table 2: Antifungal activity of Australian tea tree oil and terbinafine-HCl against different Malassezia pachydermatis strains in vitro.

		Australian tea tree oil				Terbinafine-HCL	
	strains	MIC % (v/v)	MFC % (v/v)	MIC µg/ml	MFC µg/ml	MIC µg/ml	MFC µg/ml
Clinical isolates							
Malassezia pachydermatis	B 7097	0.13	0.13	1120.0	1120.0	0.8–1.6	0.8–1.6
Malassezia pachydermatis	B 7104	0.06	0.13	560.0	1120.0	0.8	0.8
Malassezia pachydermatis	B 7124	0.13	0.13	1120.0	1120.0	0.4–0.8	04–0.8
Malassezia pachydermatis	B 7139	0.13	0.13	1120.0	1120.0	0.4	0.4
Malassezia pachydermatis	B 7148	0.13	0.13	1120.0	1120.0	0.8	0.8
Certificated strains							
Malassezia pachydermatis	NCPF 3595	0.06	0.06–0.13	560.0	560.0–1120.0	0.2	0.2
Malassezia pachydermatis	NCPF 3596	0.06	0.06	560.0	560.0	0.2	0.2
Malassezia pachydermatis	NCPF 3667	0.06–0.13	0.13	560.0–1120.0	1120.0	0.8	0.8
Malassezia pachydermatis	DSM 6172	0.06–0.13	0.06–0.13	560.0–1120.0	560.0–1120.0	0.8	0.8

µg/ml, respectively. As expected the susceptibility of the tested yeasts to terbinafine-HCl was prominent. MIC and MFC values ranged from 0.2 to 0.8 µg/ml terbinafine-HCl. Moreover it was shown that TTO was more active towards *Malassezia pachydermatis* compared to its lower activity to *Candida* species (e.g. *C. albicans, C. famata, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis;* MIC-values: 0.25–0.5%). In this study the MIC values often corresponded to the MBC data, and this may be indicative for a fungicidal effect of the tested TTO. The fungicidal activity of terbinafine-HCl and its killing mode of action to a wide spectrum of yeasts and dermatophytes is commonly known and supported by the presented findings (Arzeni et al., 1998; Jessup et al., 2000). Only a few data have been published on the *in vitro* susceptibility of fungi to TTO using various methods that were standardized methods. In a similar research work MICs for *Malassezia furfur* were obtained ranging from 0.12 to 0.25% (v/v) (Hammer et al., 1997). The range of the antifungal properties of TTO against these type of yeast seems to be comparable to data presented here.

Conclusions

In view of the fact that there is a clinical relevance of *Malassezia pachydermatis* in a number of animals' skin lesions the presented data provide a basis for a new rationale for using TTO in the veterinary medicine. The clear advantage of this essential oil apart from its antimycotic effects is its remarkable antibacterial activity especially against staphylococci species (Harkenthal et al., 1999). With the topical application of only one active substance, namely tea tree oil, two possible microorganisms both involved in dermatitis may be inhibited at the same time *in vitro.* Therefore clinical trials are needed to proof its efficacy *in vivo* in domestic animals. The last ten to fifteen years the use of TTO containing human health care products, cosmetics and household disinfectants increased a lot and its beneficial effects led it to a valuable addition to the synthetic antimicrobial and antiseptic substances (Saller et al., 1998). On the other hand, undiluted tea tree oil has been reported to show undesirable side effects such as allergic contact dermatitis or local skin irritations. Therefore, it is recommended to use tea tree oil preferably in formulations (e.g. creams, gels, ointments, oil of almonds) containing 5 to 10% of the essential oil (Reichling et al., 2001).

Based on this study it seems to be possible that application of TTO may develop into a promising alternative agent in the veterinary field. Its future perspective regarding topical treatment of bacteria and yeasts associated with inflammatory skin diseases in domestic animals remains to be demonstrated.

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Effet antifongique de l'huile de théier australien sur *Malassezia pachydermatis* isolé de chiens atteints de maladies de la peau

La levure *Malassezia pachydermatis* est une composante de la flore normale de la peau chez les organismes à sang chaud. Dans une série d'articles de synthèse, la participation de cette levure au développement de plusieurs maladies de la peau, particulièrement chez le chat et le chien comme par exemple la dermatite séborrhéique, est évoquée. L'activité antimycotique de l'huile de théier australien, l'huile éthérée de *Melaleuca alternifolia,* a été examinée *in vitro* contre plusieurs souches de *Malassezia pachydermatis* en tant qu'alternative aux antimycotiques et antiseptiques synthétiques utilisés couramment pour le traitement de maladies infectieuses de la peau superficielles. Toutes les souches testées ont démontré une sensibilité remarquablement élevée à l'égard de cette huile. A la suite de ces résultats, l'excellente activité antimicrobienne de l'huile de théier australien a pu être confirmée et élargie à un microorganisme pathogène qui colonise la peau des organismes à sang chaud. Au cours des 10 dernières années, l'huile de théier australien a obtenu une grande popularité en tant que composante de produits pour la santé humaine. Les résultats présentés entrouvrent pour cette huile des possibilités d'application également en médecine vétérinaire, par exemple comme alternative prometteuse aux antimycotiques synthétiques lors d'application extérieure.

Effetto antifungale dell'olio dell'albero da tè australiano sulla *Malassezia pachydermatis* isolata in cani affetti da malattie cutanee

Il saccaromicete *Malassezia pachydermatis* fa parte della normale flora cutanea nella maggior parte degli animali a sangue caldo. In una serie di lavori riassuntivi viene richiamata l'attenzione sul fatto che questo saccaromicete è implicato nella formazione di diverse malattie cutanee, specialmente nei gatti e nei cani, come ad esempio la dermatite seborroica. Quale alternativa agli antimicotici e antisettici sintetici che di solito vengono usati per il trattamento di malattie cutanee infettive superficiali, è stata esaminata *in vitro* l'attività antifungale dell'olio dell'albero da tè e più precisamente dell'olio eterico della *Melaleuca alternifolia* contro diversi ceppi di *Malassezia pachydermatis.* Tutti i ceppi esaminati hanno mostrato una grande sensitività verso l'olio dell'albero da tè. Tramite questo risultato è stata confermata l'ottima azione antimicrobica dell'olio dell'albero da tè. Inoltre l'azione antimicrobica è stata estesa ad un ulteriore agente patogeno che colonizza soprattutto la pelle degli animali a sangue caldo. Negli ultimi dieci anni l'olio dell'albero da tè è diventato molto popolare nella medicina umana quale componente di prodotti per la salute. I risultati presentati aprono nuovi sbocchi per l'utilizzo dell'olio dell'albero da tè nella medicina veterinaria, ad esempio come alternativa molto promettente a prodotti micotici sintetici per l'applicazione esterna.

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