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Antifungal activity of selected *Malassezia* indolic compounds detected in culture

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Abstract

Background

Malassezia yeasts produce bioactive indolic substances when grown on L-tryptophan agar. A panel of these substances was tested against commensal and opportunistic fungi, the Minimum Inhibitory Concentration (MIC) was determined and the potential for *in loco* antifungal activity on the skin was assessed.

Materials and Methods

Eight indoles were included (malassezin, pityriacitrin, indirubin, indolo[3,2b]carbazole, formyl-indolo[3,2 b]carbazole, tryptanthrin, 6-hydroxymethyl-indolo[3,2-b]carbazole and 6-methyl-indolo[3,2-b]carbazole) and were tested against 40 fungal strains [yeasts: *Malassezia* spp.(N=9); *Cryptococcus* spp.(N=10); *Candida* spp.(N=7); *Yarrowia lipolytica*(N=1); *Exophiala dermatitidis*

(N=2); molds: *Aspergillus* spp.(N=7); *Fusarium* spp.(N=2); *Rhizopus oryzae*(N=2)].

The concentration of 5/8 of the tested indoles on diseased skin was calculated from published data. Kruskal-Wallis and U-Mann-Whitney tests were employed for group susceptibility evaluation in 33 strains.

Results

The MIC range was 0.125-32 μ g/ml and the median log₂MIC was 4. Indirubin was the most potent antifungal agent and differed significantly from the others. The highest median MIC was found for FICZ.

Malassezia with *Candida* strains were more susceptible compared to *Cryptococcus* and *Aspergillus* and this inhibitory activity was predicted to be valid also on human skin.

Conclusions

Malassezia yeasts produce indolic species that inhibit an array of clinically significant yeasts and molds.

Introduction

The human skin harbors an array of microbial species and microorganisms with *Malassezia* yeasts being the principal eukaryotic organisms.^{1,2} This genus currently includes 17 species³ and in humans has been associated with the development of pityriasis versicolor (PV), dandruff (DF)/seborrheic dermatitis (SD) and certain head and neck manifestations of atopic dermatitis.⁴ All these common skin conditions are characterized by a variably compromised skin barrier.^{5,6}

Initially it was reported that *M. furfur* strains have the ability to produce indolic substances when grown in a medium with L-tryptophan as the exclusive nitrogen source.⁷ Subsequently, it was found that under the aforementioned culture conditions *M. furfur* strains isolated from SD and PV lesions have the ability to synthesize *in vitro* significantly larger quantities of indoles compared to those isolated from healthy skin.⁸ Furthermore, it has become clear that this property is not restricted to *M. furfur* but is wider distributed among more species within the genus, while *Malassezia* and their respective indoles can be also traced in skin scales from SD and PV patients.⁹ Important molecules of this group include malassezin, indirubin, indolo-[3,2b]-carbazole (ICZ), pityriacitrin¹⁰ and formyl-indolo-[3,2b]-carbazole (FICZ). Among potential biological effects of these substances preliminary data indicate that indirubin possesses some antifungal¹¹ and antiparasitic¹² activity, whilst it could also potentiate the antibacterial effect of ciprofloxacin against resistant *S. aureus* strains *in vitro*.¹³ Moreover, pityriacitrin has a small, yet measurable, sunlight protective factor (SPF: 1.7) at 5% concentration¹⁴ and FICZ is considered a candidate endogenous aryl-hydrocarbon receptor (AhR) ligand and an ultraviolet damage mediator on human skin.¹⁵ Regarding indirubin it mediates functions as inflammation and tumorigenesis¹⁶ and is currently suggested as a potent topical treatment for psoriasis.¹⁷ The production of these ligands from L-tryptophan is not restricted to yeasts of the *Malassezia* genus but has also been described in the ascomycetous gastrointestinal truck commensal *C. glabrata* underscoring the wider biological significance of this pathway.¹⁸

Aim of this study was to screen indoles detectable in *Malassezia* cultures for determining antifungal activity against a selection of commensal and opportunistic pathogenic fungi. The determined MIC values in µg/ml were interpreted with regard

to the concentrations of the respective indoles in skin scales in order to predict potential *in loco* antifungal activity of *Malassezia* related indoles.

Materials and Methods

Forty fungal strains maintained at the Hellenic Collection of Pathogenic Fungi-UOA/HCPF were tested against 8 *Malassezia* indoles (Table 1) including the 5 indoles previously isolated from diseased skin (malassezin, pityriacitrin, ICZ, indirubin, FICZ) and 3 additional ones tryptanthrin, 6-hydroxymethyl-indolo [3,2-b] carbazole (H) and 6-methyl-indolo [3,2-b] carbazole (M) (Table 2). The tested strains included 27 yeasts comprising *Malassezia* spp. (N=9); *Cryptococcus* spp. (N=10); *Candida* spp. (N=7); *Yarrowia lipolytica* (N=1); the black yeast *Exophiala dermatitidis* (N=2); and 13 molds *Aspergillus* spp. (N=7); *Fusarium* spp. (N=2); *Rhizopus oryzae* (N=2). The used indoles were synthesized as previously^{8,9,10} and were maintained in 10⁻²M dimethyl sulfoxide stock solutions. For the broth microdilution method the CLSI M27-A3 and M38-A2 guidelines for yeasts and molds respectively were followed. Each strain was tested twice and on each testing occasion the quality control and reference strains respectively (*Candida parapsilosis* ATCC90018 and *Candida albicans* ATCC90028 for yeasts, and for molds the quality control and reference strains respectively *Paecilomyces variotii* ATCC MYA-3630 and *Aspergillus fumigatus* ATCC MYA-3626) were included. If the results for the quality control strains differed >2 dilutions the experiment was discarded. Regarding the results from the tested fungal strains, the higher MIC of the 2 experiments is recorded in Table 1. This was performed in order to compensate for likely dilution issues of the tested indoles to avoid underscoring the MIC value.

The concentration of the applied indoles on human skin was calculated from data published previously.⁹ As the skin extracts mostly consist of skin lipids the density was taken at 0.9 gr/ml. For the transformation of the concentration of indoles in the skin extracts to corresponding µg/ml the following formula was employed:

$$Cx \text{ (mol/mg extract)} * \text{Molecular weight} * 10^9 / 0.9 \text{ (g/ml extract)} = Cx \text{ (}\mu\text{g/ml)}$$

Statistical analysis

In the statistical analysis the results from 33 strains were included as the yeast *Y. lipolytica* and the molds *Rhizopus*, *Fusarium* and *Exophiala* were excluded due to the small number of strains tested. The group susceptibility of each of the indoles was evaluated with the Kruskal-Wallis and U Mann-Whitney tests employing the SPSS software (v22, Chicago, IL, USA

Results

Table 1 compiles the MIC values for the strains of the fungal species tested. Over all tested yeast strains and indolic substances median \log_2 MIC was 4 (Fig. 1). Based on the presently employed array of fungal strains, from the indolic substances tested, indirubin (median \log_2 MIC=3) was the most potent antifungal agent and the only one that differed significantly in activity from any of the other ones: 6-methyl-indolo [3,2-b] carbazole (p=.015), malassezin (p=.004), pityriacitrin (p=.003), 6-hydroxymethyl-indolo [3,2-b] carbazole (p=.003) and FICZ (p<.001). On the other hand the highest median MIC (\log_2 MIC=5) was found for FICZ (Fig. 1).

Regarding susceptibility by fungal genera two groups were discerned: *Malassezia* with *Candida* and *Cryptococcus* with *Aspergillus*. The former group was significantly more sensitive as compared to the latter group over all tested indoles

(Figure 1; Panels A, C). The pattern of susceptibility was equivalent for each genus for pityriacitrin, FICZ and 6-hydroxymethyl-indolo [3,2-b] carbazole (Figure 1).

The concentration of the *Malassezia* associated indoles isolated from lesional skin scales were transformed to $\mu\text{g/ml}$ (Table 2) in order to compare the above susceptibility data to possible antifungal activity on the corresponding skin surface. From the 9 skin scales extracts 3 (2SD; 1PV) contain indole concentrations that would inhibit all fungal strains, including the *Malassezia* strains tested in this study. In two cases (SD2 and PV2) the respective inhibitory activity could be attributed to the indirubin concentration and in one to pityriacitrin (SD3).

Discussion

Herein we demonstrate that indolic molecular species isolated from *Malassezia* cultures have antifungal activity *in vitro* at concentrations detected in scale probes selected from lesional SD and PV skin. From the indolic substances tested, indirubin is significantly the most active one. This molecule is the target of intense investigation as it possesses anti-inflammatory and anticancer properties and it is the active ingredient of indigo naturalis, a popular Chinese traditional medicine.¹⁶ As already mentioned it is evaluated as a topical agent in the treatment of psoriasis¹⁷ and the antifungal properties detected herein could be an additional indication. Furthermore, low water solubility that characterizes indirubin could be an asset on the skin as it would not be easily removed from sweat. Strict, numerical comparison of the MIC with established antifungals¹⁹ of indirubin or the other indoles should not be performed as the antifungal function would be performed *in loco* on the skin and not after systemic intake in blood or tissue level. Thus, within the context that they are presently assessed, they do achieve on the skin concentrations that could be

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active *in vivo* against commensal (*Malassezia* and *Candida*), opportunistic pathogenic yeasts (*Cryptococcus*) and molds (*Aspergillus*). Furthermore, the existence of a synergistic action that adds to this effect cannot be excluded.

A notable observation is that they show antifungal activity against their own producers, i.e. *Malassezia* and *Candida* species. The indole concentration achieved *in vitro* by *Malassezia* species when grown on L-tryptophan agar are 2-3 dilutions lower than the herein recorded MIC.⁸ This means that production of indoles *in vitro* does not inhibit *Malassezia* growth up to a point, yet the toxicity of these substances might explain the preferential use of other nitrogen sources as is glycine and resolution to L-tryptophan use when these sources have been exhausted.²⁰ As for *Candida* species, only *C. glabrata* has been found to produce indolic substances and from these, only tryptanthrin (Mexia and Magiatis unpublished data).

Another issue underscored by the findings of this study is the exact role of *Malassezia* in the accumulation of indolic compounds in association with yeast colonies *in vivo* and *in vitro*.⁹ Most probably, on the skin the production of indolic compounds represents an adaptive phenomenon of certain *Malassezia* stains that can exploit their physico-chemical milieu in order to improve their survival competence by optimizing the bioenergetics of the assembly and accumulation of bioproducts in their environment. It has been proposed that the formation of these substances begins with an enzyme-mediated formation of indolepyruvate from L-tryptophan and the subsequent 'avital' transformation of the precursor substance into a whole array of further indolic compounds.²¹ Accordingly, malassezin can easily transform to ICZ²² and additional tryptophan metabolites can be formed on the skin through the action of the omnipresent UV radiation or further oxidative reactions.²³ Taken all these observations together, we would like to suggest that the decisive trait that

controls the variation in the capacity of different *Malassezia* strains to accumulate indolic compounds in their environment is their ability to uptake and metabolize available L-tryptophan into indolepyruvate and to excrete it into the environment where subsequent formation of further molecular species continues depending on the available milieu conditions. Hence, effective antifungal concentrations of these substances might not be spatially related to the *Malassezia* microcolonies on the skin, thus not actually inhibiting the producer strains.

In conclusion, *Malassezia* yeasts produce indolic species that have the ability to inhibit an array of yeast and mold strains at clinically meaningful concentrations. Future research should elaborate on the clinical significance of this observation by expanding our knowledge on the distribution of these bioactive substances on healthy and diseased skin.

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Conflict of Interest

None

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Table 1. The fungal species included in the study and the corresponding Minimum Inhibitory Concentrations (MIC) of the 4 *Malassezia* indoles tested ($\mu\text{g/ml}$).

		Malassezin	ICZ	Pityriacitrin	Indirubin	FICZ	6-hydroxy-methyl-indolo[3,2-b]carbazole	Tryptanthrin	6-methyl-indolo[3,2-b]carbazole
Yeasts	<i>Candida albicans</i> ATCC90028	8	8	8	8	32	16	8	16
	<i>Candida albicans</i> CBS 562	0.125	4	4	0.5	32	0.0125	2	16
	<i>Candida glabrata</i> CBS 7904	8	8	16	4	32	8	8	8
	<i>Candida krusei</i> ATCC6258	8	4	16	4	16	8	4	8
	<i>Candida parapsilosis</i> ATCC90018	8	8	8	8	32	16	8	8
	<i>Candida tropicalis</i> CBS94	8	4	8	8	8	8	8	16
	<i>Candida tropicalis</i> IP 2148-93	0.032	32	32	2	32	0.25	2	8
	<i>Cryptococcus albidus</i> CR 127	32	32	32	16	32	32	32	32
	<i>Cryptococcus gattii</i> CR86	32	32	16	16	16	32	16	32
	<i>Cryptococcus laurentii</i> CR 125	32	32	16	16	32	32	32	32
	<i>Cryptococcus neoformans</i> CR192	32	32	16	16	16	32	4	32
	<i>Cryptococcus neoformans</i> UOA/HCPF 9235	32	32	32	32	32	32	32	32
	<i>Cryptococcus neoformans sero A</i> CR 12	32	32	32	16	32	16	32	8
	<i>Cryptococcus neoformans sero AD</i> CR 48	32	32	32	16	32	32	32	8
	<i>Cryptococcus neoformans sero AD</i> CR 49	32	32	32	16	32	32	32	16
	<i>Cryptococcus neoformans sero C</i> CR 81	32	32	32	16	32	32	32	32
	<i>Cryptococcus neoformans sero D</i> CR 96	32	32	32	16	32	32	32	32
	<i>Malassezia dermatis</i> CBS9145	16	4	32	4	8	16	4	16
	<i>Malassezia furfur</i> CBS 7983	16	8	16	8	16	16	8	16
	<i>Malassezia globosa</i> UOA/HCPF 15443	8	2	16	2	2	8	1	8
<i>Malassezia nana</i> CBS9559	16	8	16	8	16	8	16	16	
<i>Malassezia pachydermatis</i> CBS1880	16	8	16	8	16	16	8	8	
<i>Malassezia restricta</i> UOA/HCPF15428	16	4	4	2	4	8	16	8	
<i>Malassezia slooffiae</i> CBS7956	4	2	16	4	8	8	1	4	
<i>Malassezia sympodialis</i> CBS7222	16	16	8	4	32	16	8	16	
<i>Malassezia yamatoensis</i> CBS 9725	16	8	16	8	16	16	16	16	
<i>Yarrowia lipolytica</i> CBS6124-1	8	8	16	4	16	8	8	8	
Molds	<i>Aspergillus flavus</i> UOA/HCPF 12726	32	32	32	32	32	32	32	32
	<i>Aspergillus flavus</i> UOA/HCPF15587	16	8	8	8	8	16	8	16
	<i>Aspergillus fumigatus</i> UOA/HCPF 14662	8	16	32	16	32	4	32	8
	<i>Aspergillus fumigatus</i> UOA/HCPF 7431	32	32	32	32	32	32	32	32
	<i>Aspergillus fumigatus</i> UOA/HCPF15831	16	32	16	8	8	32	8	16
	<i>Aspergillus niger</i> UOA/HCPF 14744	32	32	32	32	32	32	32	32
	<i>Aspergillus niger</i> UOA/HCPF15749	8	4	4	8	8	8	8	4
	<i>Exophiala dermatitidis</i> UOA/HCPF 3801	32	32	32	32	32	32	32	32
	<i>Exophiala dermatitidis</i> UOA/HCPF3801	16	16	8	16	16	16	16	16
	<i>Fusarium solani</i> UOA/HCFP 2213	32	32	16	16	16	32	16	32
	<i>Fusarium oxysporum</i> UOA/HCFP 12739	32	32	32	32	32	32	32	32
	<i>Rhizopus oryzae</i> UOA/HCPF 3908	32	32	32	32	32	32	32	32
	<i>Rhizopus oryzae</i> UOA/HCPF15489	8	4	8	4	8	16	8	16

Table 2. *Malassezia* associated indole concentration ($\mu\text{g/ml}$) on skin extracts and respective fungal genera that at least one strain tested would be inhibited on diseased human skin. Concentrations are adapted from⁹. PV: Pityriasis versicolor; SD: Seborrheic dermatitis; ICZ: indolo-[3,2b] –carbazole

	Malassezin	ICZ	Pityriacitrin	Indirubin	Species inhibited
SD1				23.57	<i>Candida, Cryptococcus, Malassezia</i>
SD2				43.66	<i>Aspergillus, Cryptococcus, Fusarium, Candida, Malassezia</i>
SD3	0.06		34.24		<i>Aspergillus, Cryptococcus, Fusarium, Candida Malassezia</i>
SD4				1.22	<i>Candida</i>
SD6	0.06	2.84	1.87		<i>Candida</i>
PV1		5.97	6.84		<i>Candida, Malassezia</i>
PV2	0.76			75.68	<i>Aspergillus, Cryptococcus, Fusarium, Candida, Malassezia</i>
PV3	0.33			3.78	<i>Candida, Malassezia</i>

Figure 1. Susceptibility of the 33 fungal strains included in the study. Panel A.

Susceptibility of the fungal genera included in the study against each indole. Panel B. Antifungal activity of the tested indoles against all strains of the genera included in the study i.e. *Malassezia*, *Candida*, *Cryptococcus* and *Aspergillus*. Indirubin was the most active substance and FICZ the less active one Panel B. Susceptibility of the 4 genera included in the study against the array of indoles employed. FICZ: formyl-indolo – [3,2 b] – carbazole; ICZ: indolo- [3,2b] -carbazole. The asterisk denotes statistical significance.

