In vitro activity of conventional antifungal drugs and natural essences against the yeast-like alga Prototheca

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Received 13 November 2007; returned 29 December 2007; revised 8 February 2008; accepted 20 February 2008

Background: Two outbreaks of mastitis due to the yeast-like alga Prototheca zopfii recently occurred in dairy herds in Lombardia (Italy) involving 180 and 150 lactating cows, respectively.

Objectives: To determine the in vitro susceptibility of Prototheca isolates to conventional antifungal agents and to essential oils.

Methods: Twenty P. zopfii isolated from milk during these outbreaks, six P. zopfii isolated from fresh water and two Prototheca sp. reference strains were submitted to antifungal susceptibility testing by broth microdilution assay following the CLSI guidelines for yeasts.

Results: The tested isolates were shown to be resistant to fluconazole and caspofungin. A wide range of voriconazole MICs was observed. In contrast, amphotericin B, itraconazole and posaconazole appeared active with MICs ≤ 1 mg/L. Bergamot and tea tree oils seemed to exert an interesting activity against this yeast-like alga.

Conclusions: Difficulties in treating animals with conventional drugs and the potent in vitro activity of essential oils demonstrated here raise the interest in further investigations on the therapeutic use of these non-conventional natural products.

Keywords: bovine mastitis, tea tree oil, bergamot oil

Introduction

The genus Prototheca includes unicellular achlorophyllous yeast-like microalgae that reproduce by formation of a variable numbers of sporangiospores within a sporangium.1 Five species—Prototheca wickerhamii, Prototheca zopfii, Prototheca stagnora, Prototheca ulmea and Prototheca blaschkeae—have been recognized.1,2 In addition, three distinct biotypes of P. zopfii have been defined on the basis of phenotypic characteristics, and recently by molecular characterization.2,3 Prototheca species are ubiquitous and can be isolated from various environmental reservoirs, such as slime flux of trees, grass, fresh and salt water, and wastewater. A pathogenic potential has been indicated for P. wickerhamii and P. zopfii. Although the former is the predominant cause of human infections, the latter causes infections in animals, particularly in cows or dogs.3,4

P. zopfii has been identified as inducing a therapy-resistant inflammation of the mammary gland in dairy cows leading to severe losses in an infected herd.5 These algae do not respond to routine mastitis therapy and the only control measure to date has been the elimination of the infected animals.5 Two outbreaks of P. zopfii mastitis recently occurred in dairy herds in Lombardia (Italy) involving 180 and 150 lactating cows, respectively.

Recently, the potential antimicrobial effects of essential plant oils have attracted serious attention within the scientific community. Several reports have documented the antimicrobial effects of essential oils extracted from various plant species, such as Melaleuca alternifolia (tea tree) and, more recently, from Citrus bergamia (bergamot).7,8

The aim of this study was to investigate the in vitro susceptibility of Prototheca isolates to conventional antifungal agents and to essential oils.
In vitro susceptibility of Prototheca

Table 1. In vitro susceptibility profile of the Prototheca isolates and of quality control strains

<table>
<thead>
<tr>
<th></th>
<th>Prototheca (27 isolates)</th>
<th>P. wickerhamii (1 isolate)</th>
<th>C. parapsilosis ATCC 22019</th>
<th>C. krusei ATCC 6258</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM range MIC₉₀</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.46 0.25–0.5 0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>≥128 ≥128 &gt;128</td>
<td>≥128</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.46 0.12–1 1</td>
<td>0.5</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.47 0.12–1 1</td>
<td>0.25</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>1.85 0.5–32 8</td>
<td>0.5</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>&gt;16 &gt;16 &gt;16</td>
<td>&gt;16</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tea tree oil</td>
<td>0.05 0.03–0.12 0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Bergamot oil</td>
<td>1.45 0.15–5 5</td>
<td>0.3</td>
<td>1.25</td>
<td>5</td>
</tr>
</tbody>
</table>

GM, geometric mean. MIC₉₀; MIC at which 90% of the isolates were inhibited.

Materials and methods

A total of 20 P. zopfii biotype II strains isolated from milk cultured during the above-mentioned outbreaks, 6 P. zopfii biotype II strains isolated from the Po, Staffora and Lambro rivers in the 1970s and 2 reference strains (P. zopfii CDC B3086 and P. wickerhamii CDC B4553) were used for the study. The isolates were identified on the basis of the presence of dauer cells and on biochemical features (assimilation of glucose, galactose, glycerol, sucrose and trehalose, and growth at 28 and 37°C).1,3

In vitro susceptibility testing was performed by broth microdilution assay following the CLSI (formerly NCCLS) guidelines for yeasts.9 Six antifungal drugs—amphotericin B (Sigma, Milano, Italy), itraconazole (Janssen Research Foundation, Beerse, Belgium), fluconazole (Sigma), voriconazole (Molekula Ltd, Wimborne, Dorset, UK), posaconazole (Schering-Plough Research Institute, Kenilworth, NJ, USA) and caspofungin (Merck & Co., Whitehouse Station, NJ, USA)—and two essential oils—tea tree oil and bergamot (Farmacia Legnani, Milano, Italy)—were investigated. Tween 80 (final concentration 0.001%, v/v) was included to facilitate oil solubility.7 Final concentrations ranged from 0.03 to 16 mg/L for amphotericin B, caspofungin, itraconazole and posaconazole, from 0.25 to 128 mg/L for fluconazole, from 0.0078% to 4% for tea tree oil and from 0.02% to 10% for bergamot oil. Testing was performed in RPMI 1640 without sodium bicarbonate (Sigma) buffered to pH 7.0 with 0.165 M MOPS (Sigma) supplemented with 2% glucose and 0.03% L-glutamine (Sigma). Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used as quality control strains. Tests were performed in duplicate. Microplates were read visually.

The MIC (mg/L for antifungals and % for essential oils) was defined as the lowest concentration that produced a 50% reduction (100% for amphotericin B) of growth compared with control growth under drug-free conditions, after 72 h of incubation. Resistance breakpoints were those reported by the CLSI as documented or tentative for Candida spp., namely ≥64, ≥1, ≥2, ≥4, ≥2 and ≥2 mg/L for fluconazole, itraconazole, posaconazole, voriconazole, caspofungin and amphotericin B, respectively.

Results and discussion

Table 1 summarizes the in vitro susceptibility profile of the Prototheca isolates and of quality control strains to amphotericin B, itraconazole, fluconazole, voriconazole, posaconazole and caspofungin, and to tea tree oil and bergamot. Geometric mean MICs, MIC ranges and MICs at which 90% (MIC₉₀) of the strains were inhibited are reported.

Prototheca tested isolates were shown to be resistant to fluconazole (MIC ≥ 128) and caspofungin (MIC > 16). Resistance to fluconazole is well recognized and our finding confirms the results of previous investigations.4 Lack of β-glucans, the specific target of echinocandins, in the cell wall of this alga may be the reason for resistance to caspofungin. A wide range of voriconazole MICs, from 0.5 up to >16 mg/L, was observed. As already shown,4,10 the P. wickerhamii isolate was susceptible to this azole, whereas P. zopfii was less susceptible up to resistant, the MICs being ≥ 4 mg/L for seven isolates. In addition, amphotericin B and posaconazole appeared to be active in vitro with MICs ≤ 1 mg/L. Susceptibility to polyene and azole agents has been explained by the presence of ergosterol in the neutral lipid fraction of the cell membranes of Prototheca species. Bergamot and, especially, tea tree oil seemed to exert an interesting in vitro activity against this yeast-like alga.

In conclusion, despite the in vitro activity of several antifungal drugs, difficulties in treating animals with these conventional drugs and the potent in vitro activity of essential oils demonstrated here raise the interest in further investigations on the therapeutic use of these non-conventional natural products.

Funding

No specific funding was received.

Transparency declarations

None to declare.

References


