# Activity of exogenous tyrosol in combination with caspofungin and micafungin against *Candida parapsilosis* sessile cells.

Short Title: Echinocandins with tyrosol

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## Abstract

**Aims:** Fungal quorum sensing molecules may have an inhibitory effect as adjuvant against *Candida* biofilms. Therefore, *in vitro* activity of caspofungin and micafungin was evaluated against *Candida parapsilosis* biofilms in the presence of tyrosol.

**Methods and Results:** Interactions were assessed using fractional inhibitory concentration index (FICI) determination, metabolic activity-based time-kill experiments, and scanning electron microscopy. Tyrosol caused 1- to 16-fold and 2- to 32-fold decrease in median caspofungin and micafungin MICs, respectively. Based on FICI, synergy was observed in isolates 27001 and 17820 with caspofungin and 27001 with micafungin. In time-kill experiments, the metabolic activity reduction were higher for micafungin compared to caspofungin at 24 hours especially when used in 64 mg $l^{-1}$ and 256 mg $l^{-1}$ concentrations. In the case of micafungin, the 256 mg $l^{-1}$+1 mmol $l^{-1}$ combination caused significantly higher decrease in metabolic activity compared to the corresponding concentration alone (256 mg $l^{-1}$) at 24 hours ($p$<0.05). Scanning electron microscopy confirmed the higher killing effect of tested echinocandins with tyrosol.

**Conclusions:** Considerable metabolic activity reduction and cell damage was detected for combinations especially in case of micafungin.

**Significance and Impact of Study:** Our findings support the development of new alternative therapeutic strategies against *C. parapsilosis* biofilms.



Keywords: Biofilms, Quorum sensing, Echinocandins, Time-kill experiments, Synergism, *Candida parapsilosis*, SEM

## Introduction

Based on epidemiological studies, *Candida parapsilosis sensu stricto* is one of the most common fungal species causing catheter-associated infections (Puig-Asensio *et al*. 2014; Marcos-Zambrano *et al*. 2014c). Although the recommended procedure in such cases is catheter removal and replacement, antifungal lock therapy may be an alternative approach for patients with limited venous access (Mermel *et al*. 2009). Several potential lock solutions were tested *in vitro* against various *Candida* species recently; however, the number of studies focusing on *C. parapsilosis* is very limited (Walraven and Lee 2013).

Echinocandins may be effective drugs in lock therapy due to their anti-biofilm effect; however, *C. parapsilosis* is innately less susceptible to these antifungal agents (Garcia-Effron *et al*. 2008). Based on previous reports, quorum sensing molecules derived from *Candida* species (farnesol, tyrosol) may be potential adjuvants in case of various alternative treatment strategies (ie: antifungal lock therapy) (Shanmughapriya *et al*. 2014; Katragkou *et al*. 2015; Cordeiro *et al*. 2015; Monteiro *et al*. 2015; Kovács *et al*. 2016). *In vitro* synergy between echinocandins and farnesol against biofilms has been reported for both *C. albicans* and *C. parapsilosis* (Katragkou *et al*. 2015; Kovács *et al*. 2016). However, there is limited information on interactions between antifungal agents and tyrosol. Under physiological condition, tyrosol stimulates germ tube formation in yeast cells and hyphae formation in the early stage of biofilm production (Alem *et al*. 2006). Paradoxically at the same time, as described before, it may have an inhibitory effect alone or in combination with traditional antifungals on biofilm formed by *C. albicans* and *C. tropicalis* (Cordeiro *et al*. 2015; Monteiro *et al*. 2015). Nevertheless, interaction between echinocandins and tyrosol has remained unknown so far.

Hence the aim of this study was to investigate the interaction of caspofungin and micafungin in combination with tyrosol against *C. parapsilosis* biofilms using fractional inhibitory

74 concentration index (FICI) determination, metabolic activity-based time-kill experiments, and
75 scanning electron microscopy (SEM).
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## Materials and methods

### Fungal isolates

Five clinical *C. parapsilosis sensu stricto* isolates (16879, 16977, 17820, 22482, 27001) derived from haemocultures were included in this study along with reference strain ATCC 22019. Clinical isolates were identified by MALDI/TOF (Microflex, Bruker Daltronics, Bremen, Germany) and were also confirmed by molecular biology methods (Tavanti *et al*. 2005).

### Susceptibility testing of planktonic cells

Antifungal susceptibility to caspofungin (pure powder, Sigma, Budapest, Hungary), micafungin (pure powder, Astellas Pharma Inc., Tokyo, Japan) and tyrosol (Sigma, Budapest, Hungary) was investigated using broth microdilution method in RPMI-1640 (with L-glutamine and without bicarbonate, pH 7.0 with MOPS; Sigma, Budapest, Hungary) in line with the CLSI standard M27-A3 protocol (Clinical and Laboratory Standards Institute, 2008). The final concentrations of each drug ranged from 0.12 to 8 mg $l^{-1}$ for caspofungin and micafungin. Tyrosol concentrations ranged from 3.9 to 1000 μmol $l^{-1}$ (0.54 to 138.2 mg $l^{-1}$), which is approximately 1 to 250 times the endogenous tyrosol concentration reached in our *C. parapsilosis* isolates (not published data). A 50 mmol $l^{-1}$ working stock solution of tyrosol was prepared in 100% methanol. The working concentrations were prepared in RPMI-1640. Each drug-free control well contained 1% (v/v) methanol.

Susceptibility testing of planktonic cells was performed in 96-well plates (TPP, Trasadingen, Switzerland) at 37°C over 24 hours based on our previous study with farnesol (Kovács *et al*. 2016). Minimum inhibitory concentrations (MICs) were defined based on turbidity (492 nm), MIC being the lowest drug concentration capable of inhibiting 50% of fungal growth compared to the growth control well. Percentage change in turbidity was calculated on the

basis of absorbance (A) as 100% × ($A_{well} - A_{background}$)/($A_{drug\text{-}free\ well} - A_{background}$) (Katragkou *et al*. 2015; Kovács *et al*. 2016). All isolates were tested in three independent experiments, and the median of these three values was used in the analysis.

**Biofilm formation**

One-day-old biofilms were prepared as described by Pierce *et al.* (2008). Briefly, 100 µl of standardized *C. parapsilosis* suspension ($1x10^6$ CFU $ml^{-1}$) in RPMI-1640 were inoculated into each well of polystyrene, flat-bottom 96-well microtitre plates (TPP, Trasadingen, Switzerland) and incubated statically for 24 hours at 37°C. After the incubation time, plates were washed three times with physiological saline to remove unattached cells. The metabolic activity of *C. parapsilosis* biofilms was measured using XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reduction assay as described previously with minor modifications (Hawser 1996). A 100 µl aliquot of XTT/menadione solution (XTT solution (Sigma) was 0.5 g $l^{-1}$ that was supplemented with menadione (Sigma) to a final concentration of 1 µmol $l^{-1}$) was added to each well containing the preformed washed biofilms. Plates were covered with aluminium foil and were incubated in darkness for 2 hours at 37°C. After the incubation time, 80 µl of supernatant from each well was measured spectrophotometrically at 492 nm.

All clinical isolates and the reference strain were tested three times and classified according to metabolic activity cut-off values published by Marcos-Zambrano *et al.* (2014a). Accordingly, each clinical isolate had high metabolic activity whilst ATCC 22019 showed medium metabolic activity.

**Susceptibility testing of biofilms**

The tested concentrations for MIC determination in sessile cells were 8 to 512 mg $l^{-1}$ and 3.9

to 1000 μmol $l^{-1}$ for caspofungin/micafungin and tyrosol, respectively. The anti-biofilm activity of echinocandins and tyrosol was evaluated using XTT reduction assay (Hawser 1996; Katragkou *et al*. 2015; Kovács *et al*. 2016). Biofilm MICs were determined following 24 hours incubation with antifungal compounds and tyrosol. Change in metabolic activity was evaluated spectrophotometrically at 492 nm (Pierce *et al*. 2008). MIC was defined as the lowest concentration resulting in at least 50% reduction in metabolic activity of fungal biofilms compared to growth control cells. Percentage change in metabolic activity was calculated as described in the previous section. All isolates were tested in three independent experiments and the median of the three values was used in the analysis.

**Interactions between echinocandins and tyrosol**

Drug-tyrosol interactions were evaluated using FICI determination for both planktonic cells and biofilms. FICI is expressed using the following formula: $\Sigma FIC=FICA+FICB=MIC_A^{comb}/MIC_A^{alone}+MIC_B^{comb}/MIC_B^{alone}$, where $MIC_A^{alone}$ and $MIC_B^{alone}$ stand for MIC values of compounds A and B used alone and $MIC_A^{comb}$ and $MIC_B^{comb}$ represent the MICs of compounds A and B at isoeffective combinations, respectively. FICI was defined as the lowest ΣFIC (Meletiadis *et al*. 2005; Katragkou *et al*. 2015; Kovács *et al*. 2016). MIC values of the drugs alone and of all isoeffective combinations were determined as the lowest drug concentrations resulting in at least 50% reduction of turbidity for planktonic or at least 50% reduction of metabolic activity for sessile cells as compared with the untreated controls. Off-scale MICs were converted to the next highest two-fold concentration. The interactions was considered as synergistic when FICI was ≤0.5, no interaction when FICI was between >0.5 and 4, and antagonism when FICI was >4 (Meletiadis *et al*. 2005; Katragkou *et al*. 2015; Kovács *et al*. 2016).

**Metabolic activity changes of biofilms over exposure time**

Based on our previous study, the dynamic of metabolic activity change induced by echinocandins alone and in combination with tyrosol was examined using metabolic activity-based time-kill experiments (Kovács *et al.* 2016). Based on results of the checkerboard method, the drug concentrations tested were 16 mg $l^{-1}$, 64 mg $l^{-1}$, and 256 mg $l^{-1}$ of caspofungin or micafungin with or without 1 mmol $l^{-1}$ tyrosol. Biofilms were washed three times with physiological saline then the various drug concentrations in RPMI-1640 were added to each well assigned to given endpoints 3, 6, 9, 12 and 24 hours. After 3, 6, 9, 12 and 24 hours of incubation time, the corresponding wells washed three times and the metabolic activity of the drug-treated and untreated biofilms were read spectrophotometrically as described above (Kovács *et al*. 2016). Baseline metabolic activity was read from wells containing no drug at time zero. Curves were prepared from the measured metabolic activity values using GraphPad Prism 6.05.

**Scanning electron microscopy analysis**

The biofilm structure of one representative *C. parapsilosis* isolate was examined after caspofungin and micafungin treatment in the presence and absence of tyrosol. One-day-old biofilms were created on 10 mm diameter circular coverslips and prepared for SEM analyses as published previously Chandra *et al*. (2008). After 24 hours of growth, the disks were washed with physiological saline, and various drug concentrations were added to the samples (256 mg $l^{-1}$ caspofungin, 256 mg $l^{-1}$ micafungin, 1 mmol $l^{-1}$ tyrosol, 256 mg $l^{-1}$ caspofungin + 1 mmol $l^{-1}$ tyrosol, 256 mg $l^{-1}$ micafungin + 1 mmol $l^{-1}$ tyrosol). After 24 hours of antifungal treatment, biofilms were washed with sterile physiological saline and placed in 2% glutaraldehyde fixative solution. Samples were then dehydrated in a sequence of ethanol and dried in a desiccator. Eventually, they were coated with gold prior to observations (Hitachi S-

4300) (Chandra *et al*. 2008).

**Statistical analysis**

In time-kill experiments, one-way ANOVA with Dunnett's post-testing was used to analyse the reduction of metabolic activity caused by the drug alone and in combination compared to the drug-free control. Concentrations with and without tyrosol were compared with each other using t-test. Significance was defined as $p<0.05$.

# Results

### Susceptibility result for planktonic cells

The planktonic MIC values and ranges of caspofungin and micafungin alone and in combination with tyrosol are summarized in Table 1a and Table 1b. Three out of five planktonic clinical isolates and ATCC 22019 were susceptible to caspofungin while isolates 16977 and 17820 showed intermediate susceptibility based on revised CLSI breakpoints, while isolate 16977 had intermediate susceptibility for micafungin (Pfaller *et al*. 2011). The tested tyrosol concentrations had no inhibitory effect on against planktonic isolates (Table 1a, Table 1b).

### Susceptibility result for sessile cells

The median values and ranges of MICs for sessile *C. parapsilosis* cells are shown in Table 2a and Table 2b. Tyrosol caused 1- to 16-fold and 2- to 32-fold decrease in median caspofungin and micafungin MIC values, respectively. Furthermore, median tyrosol MICs showed 2- to 8-fold reduction when combined with echinocandins in case of biofilms (Table 2a, Table 2b).

### Interactions between tyrosol and echinocandins

The interactions of caspofungin-tyrosol and micafungin-tyrosol based on FICI are summarized in Table 1a and Table 1b for planktonic cells, and in Table 2a and Table 2b for biofilms. No antagonism was observed in either planktonic, or sessile cells (FICIs <4) (Table 1a, Table 1b, Table 2a, Table 2b). For all planktonic isolates, there were no interactions (Table 1a, Table 1b). In case of biofilms, the mean FICI varied relatively more widely both for caspofungin (range of mean FICI: 0.298-1.02) and micafungin (range of mean FICI: 0.193-1.334), respectively. Synergy was observed in isolates 27001 and 17820 for

caspofungin and 27001 for micafungin.

**Assessment of metabolic activity change over time**

Based on time-kill experiments, the effect of caspofungin and micafungin alone and combined with tyrosol on the metabolic activity of biofilms was concentration-dependent. Having the various isolates behaved similarly, all five individual *C. parapsilosis* clinical isolates included in a single plot for both caspofungin and micafungin. In case of caspofungin, significant metabolic activity reduction was observed between 3 hours and 12 hours for all tested combinations. However, there were no significant differences between the tested concentrations alone and the corresponding combinations (16 mg $l^{-1}$ vs. 16 mg $l^{-1}$ + 1 mmol $l^{-1}$, etc.) ($p$>0.05) (Fig. 1a). No significant reduction was observed with any caspofungin concentrations (neither alone nor in combinations) at 24 hours compared to the control ($p$>0.05) (Fig. 1a).

In case of micafungin, significant decrease of metabolic activity was detected between 3 hours and 24 hours regardless of the concentration or combination ($p$<0.05-0.001) (Fig. 1b). Notably, when 256 mg $l^{-1}$ micafungin was used in combination with 1 mmol $l^{-1}$ tyrosol, 91.4±3.84% reduction of metabolic activity was detected at 24 hours. In addition, the 256 mg $l^{-1}$ + 1 mmol $l^{-1}$ combination caused significantly higher decrease in metabolic activity compared to the corresponding concentration alone (256 mg $l^{-1}$) at 24 hours ($p$<0.05) (Fig. 1b).

**Scanning electron microscopy**

Isolate 16977 was randomly chosen to evaluate with SEM as representative isolate. The untreated control biofilm displayed yeast cell aggregates with smooth cell surface and few pseudohyphae (Fig. 2a). Tyrosol-exposed biofilm (1 mmol $l^{-1}$) displayed more bud scars as

well as denser structure as compared with untreated biofilm (Fig. 2b). After exposure to 256 mg $l^{-1}$ caspofungin, enlarged round blastospores with wrinkled surface were observed but appeared unaffected cells as well (Fig. 2c). Treatment with 256 mg $l^{-1}$ micafungin has resulted similar pattern to caspofungin-treated cells (Fig. 2e). Combined treatment with echinocandins and tyrosol (256 mg $l^{-1}$ caspofungin + 1 mmol $l^{-1}$ tyrosol and 256 mg $l^{-1}$ micafungin + 1 mmol $l^{-1}$ tyrosol) resulted more significant cell damage (Fig. 2d, Fig. 2f). Numerous collapsed yeast cells and blastospores with abnormal morphology were detected both for caspofungin + tyrosol and micafungin + tyrosol. These microscopic differences were confirmed by metabolic activity-based time-kill assay (Fig. 1a, Fig. 1b).

## Discussion

Over the last few years, many studies have shown that fungal quorum sensing molecules alone or in combination with certain antifungal agents have a potential inhibitory effect against *Candida* biofilms (Shanmughapriya *et al*. 2014; Katragkou *et al*. 2015; Cordeiro *et al*. 2015; Monteiro *et al*. 2015; Kovács *et al*. 2016) Most reports focus on the effect of farnesol against sessile *Candida* cells. Therefore there is limited information available on tyrosol, which is known to have opposite effect compared with farnesol under physiological condition (Alem *et al*. 2006).

Cordeiro *et al.* (2015) showed that exogenous tyrosol in supraphysiological concentration (2.5-5 mmol $l^{-1}$) significantly inhibits the growth of planktonic *C. albicans* and *C. tropicalis* isolates. Present study has found no inhibitory effect of tyrosol against *C. parapsilosis* planktonic cells. However, the highest tested tyrosol concentration was much lower than what was used by Cordeiro *et al*. (1 mmol $l^{-1}$ vs. 22 mmol $l^{-1}$). Furthermore, Cordeiro *et al.* (2015) reported synergistic interactions of tyrosol with amphotericin B and azoles against *C. albicans* and *C. tropicalis* planktonic cells based on FICI determination. Unlike these findings, current study using tyrosol combined with caspofungin or micafungin against *C. parapsilosis* planktonic cells has not found synergistic interactions.

Previous studies suggested that exogenous quorum sensing molecules were able to enhance the activity of various traditional antifungal drugs against *Candida* biofilms. Katragkou *et al.* (2015) observed clear synergism between farnesol and micafungin or fluconazole. Our previous work has shown that farnesol enhances the *in vitro* activity of caspofungin and micafungin against *C. parapsilosis* biofilms (Kovács *et al*. 2016). Cordeiro *et al.* (2015) observed that exogenous tyrosol significantly reduced biofilms of *C. albicans* and *C. tropicalis* both alone and in combination with azoles and amphotericin B against early stage of biofilms.

Based on FICI described in this study, synergism was observed in two out of six and one out of six sessile isolates for caspofungin (mean FICI 0.298-0.312) and micafungin (mean FICI: 0.193), respectively. However, it is noteworthy that FICI determination has some disadvantages that may cause difficulties in interpretation of interactions (e.g.: dilution-based determination of MICs may lead to interexperimental errors, the choice of MIC end point is not clear, FICI is not applicable for statistical analysis etc.) (Mukherjee *et al*. 2005).

In metabolic activity-based time-kill experiments, significant metabolic activity decrease was observed with micafungin in all tested concentrations and combinations between 3 and 24 hours for sessile cells. Additionally, the 256 mg $l^{-1}$ micafungin with tyrosol was statistically superior compared to 256 mg $l^{-1}$ alone at 24 hours ($p<0.05$). In contrast, significant metabolic activity reduction was observed only in the first 12 hours with various caspofungin and tyrosol combinations. In addition, only 256 mg $l^{-1}$ caspofungin without tyrosol was able to trigger significant metabolic activity decreases in the first 12 hours ($p<0.001$). Micafungin with tyrosol was much more active against *C. parapsilosis* biofilms than caspofungin with tyrosol in terms of metabolic activity. The lack of data dealing with interaction of echinocandins and tyrosol in the literature has precluded comparisons.

Although the XTT-based assays are good predictors of the effect of various antimicrobial compounds, they may have some limitations (e.g.: do not always ensure equivalence with cell death). Nonetheless, our metabolic activity-based time-kill results were in line with SEM images especially for micafungin + tyrosol where the incidence of cell damages was higher in biofilms. It should be pointed out that all tested clinical *C. parapsilosis* isolates without treatment showed high metabolic activity based on cut-off values described by Marcos-Zambrano *et al*. (2014a). Notably, micafungin is more active against biofilms with high metabolic activity at least in case of *C. albicans* (Marcos-Zambrano *et al*. 2014b). Furthermore, enhanced micafungin activity was observed against *C. parapsilosis* biofilm

earlier by Shirazi and Kontoyiannis (2015). On the other hand, Quindós *et al*. (2009) observed that the activity exerted by micafungin is highly variable against *C. tropicalis* and *C. parapsilosis* biofilms.

In conclusion, this is the first study to evaluate *in vitro* interaction of tyrosol and echinocandins against *C. parapsilosis* biofilms. No antagonistic effect was observed when tyrosol was used in combination with various echinocandins. In addition, based on time-kill findings and SEM images, considerable metabolic activity reduction and cell damage was detected especially in case of micafungin. This knowledge may result the development of new biofilm-related alternative treatments where tyrosol may be an adjuvant anti-biofilm agent.

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## Conflict of interest

L. Majoros received conference travel grants from MSD, Astellas and Pfizer. All other authors declare no conflicts of interest.

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390

**Table 1** Minimum inhibitory concentrations (MICs) of caspofungin (a) and micafungin (b) alone and in combination with tyrosol against *Candida parapsilosis* planktonic cells.

**a**

| Isolate (Planktonic) | Median MIC (range) of drug used | | | | FICI Mean (range) | Type of interaction |
|---|---|---|---|---|---|---|
| | Alone | | In combination | | | |
| | Caspofungin (mg $l^{-1}$) | Tyrosol (µmol $l^{-1}$) | Caspofungin (mg $l^{-1}$) | Tyrosol (µmol $l^{-1}$) | | |
| **ATCC 22019** | 2 | >1000[1] | 1 | 1000 | 1 | No interaction |
| **27001** | 2 (2-4) | >1000[1] | 2 (2-4) | 3.9 (3.9-7.8) | 1.003 (1.002-1.004) | No interaction |
| **17820** | 4 (2-4) | >1000[1] | 2 (2-4) | 15.62 (3.9-62.5) | 0.847 (0.508-1.031) | No interaction |
| **16977** | 4 (2-4) | >1000[1] | 4 (2-4) | 3.9 (3.9-62.5) | 1.011 (1.002-1.031) | No interaction |
| **22482** | 2 | >1000[1] | 1 (1-2) | 31.25 (31.25-62.5) | 0.687 (0.515-1.031) | No interaction |
| **16879** | 2 | >1000[1] | 2 | 7.8 (3.9-7.8) | 1.003 (1.002-1.004) | No interaction |

**b**

| Isolate (Planktonic) | Median MIC (range) of drug used | | | | FICI Mean (range) | Type of interaction |
|---|---|---|---|---|---|---|
| | Alone | | In combination | | | |
| | Micafunign (mg $l^{-1}$) | Tyrosol (µmol $l^{-1}$) | Micafungin (mg $l^{-1}$) | Tyrosol (µmol $l^{-1}$) | | |
| **ATCC 22019** | 1 (1-2) | >1000[1] | 2 | 1000 | 2.5 (1.5-2.5) | No interaction |
| **27001** | 2 (2-4) | >1000[1] | 2 | 62.5 (15.62-125) | 0.7 (0.531-1.008) | No interaction |
| **17820** | 2 (2-4) | >1000[1] | 2 (1-2) | 125 (31.25-1000) | 0.859 (0.562-1.015) | No interaction |
| **16977** | 4 | >1000[1] | 2 | 500 (250-1000) | 0.755 (0.516-1) | No interaction |
| **22482** | 2 | >1000[1] | 1 (1-2) | 1000 | 1.166 (1-1.5) | No interaction |
| **16879** | 2 | >1000[1] | 1 | 1000 | 1 | No interaction |

[1]MIC is offscale at >1000 µmol $l^{-1}$, 2000 µmol $l^{-1}$ (one dilution higher than the highest tested concentration) was used for analysis

**Table 2** Minimum inhibitory concentrations (MICs) of caspofungin (a) and micafungin (b) alone and in combination with tyrosol against *Candida parapsilosis* biofilms.

**a**

| Isolate (Biofilm) | Median MIC (range) of drug used | | | | FICI Mean (range) | Type of interaction |
|---|---|---|---|---|---|---|
| | Alone | | In combination | | | |
| | Caspofungin (mg $l^{-1}$) | Tyrosol (μmol $l^{-1}$) | Caspofungin (mg $l^{-1}$) | Tyrosol (μmol $l^{-1}$) | | |
| **ATCC22019** | 1 | >1000[2] | 1 | 3.9 | 1.002 | No interaction |
| **27001** | 256 (256-512) | >1000[2] | 32 (16-256) | 250 (3.9-250) | 0.312 (0.185-0.502) | Synergism |
| **17820** | 128 (64-128) | >1000[2] | 8 (8-16) | 250 (3.9-250) | 0.298 (0.25-0.375) | Synergism |
| **16977** | 128 (128-512) | >1000[2] | 64 (16-256) | 250 (7.8-500) | 0.584 (0.504-0.625) | No interaction |
| **22482** | 512 (512- >512) | >1000[2] | 32 (32-128) | 1000 | 0.582 (0.56-0.625) | No interaction |
| **16879** | 128 (128-256) | >1000[2] | 128 (8-128) | 1000 | 1.02 (0.560-1.5) | No interaction |

**b**

| Isolate (Biofilm) | Median MIC (range) of drug used | | | | FICI Mean (range) | Type of interaction |
|---|---|---|---|---|---|---|
| | Alone | | In combination | | | |
| | Micafungin (mg $l^{-1}$) | Tyrosol (μmol $l^{-1}$) | Micafungin (mg $l^{-1}$) | Tyrosol (μmol $l^{-1}$) | | |
| **ATCC22019** | 1 | >1000[2] | 1 | 3.9 | 1.002 | No interaction |
| **27001** | 512 (512->512) | >1000[2] | 32 | 250 (31.25-500) | 0.193 (0.049-0.375) | Synergism |
| **17820** | >512[1] | >1000[2] | 256 (32- >512) | 500 (500->1000) | 1.011 (0.5-2.002) | No interaction |
| **16977** | >512 (512->512)[1] | >1000[2] | 512 (256->512) | 1000 (1000->1000) | 1.334 (1-2.002) | No interaction |
| **22482** | >512 (512->512)[1] | >1000[2] | 128 (64-128) | 1000 (500-1000) | 0.561 (0.31-0.75) | No interaction |
| **16879** | >512[1] | >1000[2] | 32 | 500 (500-1000) | 0.53 | No interaction |

[1]MIC is offscale at >512 mg $l^{-1}$, 1024 mg $l^{-1}$ (one dilution higher than the highest tested concentration) was used for analysis

[2]MIC is offscale at >1000 μmol $l^{-1}$, 2000 μmol $l^{-1}$ (one dilution higher than the highest tested concentration) was used for analysis

**Figure 1**

Metabolic activity changes over exposure time exerted by caspofungin (a) and micafungin (b) alone and in combination with tyrosol against five sessile *Candida parapsilosis* clinical isolates. Each time point represent mean±SEM (standard error of mean) of metabolic activity of five clinical isolates.

**Figure 2**

Scanning electron microscopic images of preformed *Candida parapsilosis* clinical isolate 16977. Biofilms were grown on circular coverslip for 24 hours prior to treatment with different agents (a). Afterwards, the treated disks were incubated for 24 hours in RPMI-1640 containing 1 mmol $l^{-1}$ tyrosol alone (b), 256 mg $l^{-1}$ caspofungin (c), 256 mg $l^{-1}$ micafungin (e), 256 mg $l^{-1}$ caspofungin + 1 mmol $l^{-1}$ tyrosol (d) or 256 mg $l^{-1}$ micafungin + 1 mmol $l^{-1}$ tyrosol (f). All images represent typical fields of view. Scale bars, 20 μm.