Clinical and Microbiological Characteristics of *Candida guilliermondii* and *Candida fermentati*

Running title: Characteristics of *C. guilliermondii* and *C. fermentati*

Tatsuro Hirayama[a,b], Taiga Miyazaki[b,c]#, Yuka Yamagishi[d], Hiroshige Mikamo[d], Takashi Ueda[e], Kazuhiko Nakajima[e], Yoshio Takesue[e], Yoshitsugu Higashi[f], Yoshihiro Yamamoto[f], Muneyoshi Kimura[g], Hideki Araoka[g], Shuichi Taniguchi[h], Yuichi Fukuda[i], Yumi Matsuo[j], Akiko Furutani[k], Kohei Yamashita[a], Takahiro Takazono[b,c], Tomomi Saijo[b], Shintaro Shimamura[b], Kazuko Yamamoto[b], Yoshifumi Imamura[b], Koichi Izumikawa[c], Katsunori Yanagihara[l], Shigeru Kohno[b], Hiroshi Mukae[a,b]

[a]Department of Respiratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[b]Second Department of Internal Medicine, Nagasaki University Hospital, Nagasaki, Japan

[c]Department of Infectious Diseases, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

[d]Department of Clinical Infectious Diseases, Aichi Medical University, Japan

[e]Department of Infection Control and Prevention, Hyogo College of Medicine, Japan

[f]Department of Clinical Infectious Diseases, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Japan

[g]Department of Infectious Diseases, Toranomon Hospital, Japan

[h]Department of Hematology, Toranomon Hospital, Japan

[i]Department of Respiratory Medicine, Sasebo City General Hospital, Japan

[j]Department of Laboratory Medicine, Sasebo City General Hospital, Japan

[k]Department of Laboratory Medicine, Sasebo Kyosai Hospital, Japan

[l]Department of Laboratory Medicine, Nagasaki University Hospital, Nagasaki, Japan

#Address correspondence to Taiga Miyazaki, taiga-m@nagasaki-u.ac.jp

1-7-1 Sakamoto, Nagasaki 852-8501, Japan; Tel: +81 95 819 7273; Fax: +81 95 849 7285

Abstract

The 46 clinical isolates of *Candida guilliermondii* and *Candida famata* were re-identified genetically, resulting in 27 *C. guilliermondii* and 12 *Candida fermentati* strains. The majority of *C. guilliermondii*, but no *C. fermentati*, were isolated from blood cultures. *C. fermentati* was more sensitive to antifungals, hydrogen peroxide and killing by murine macrophages than *C. guilliermondii*. The *C. guilliermondii* isolates were echinocandin susceptible in vitro but resistant to micafungin in a murine model of invasive candidiasis.

Text

The *Candida guilliermondii* complex is a genetically heterogeneous complex of several phenotypically indistinguishable species, including *C. guilliermondii*, *Candida fermentati*, *Candida carpophila*, and *Candida xestobii* (1). The incidence of candidemia due to the *C. guilliermondii* complex ranges from 1–3% depending on the geographic region (2, 3). However, there have been limited studies reporting the epidemiological and clinical information of *C. guilliermondii* complex infections (4-6). The *C. guilliermondii* complex and *Candida famata* share similarities in biochemical characteristics, and it has been reported that these species are sometimes misidentified in clinical laboratories (7,

8).

During a 12-year period from April 2005 to March 2017, 46 strains isolated from 46 patients across seven medical institutions in Japan. All isolates were originally identified as *C. guilliermondii* or *C. famata* using standard laboratory identification systems: VITEK 2 system (BioMérieux, Marcy L'Etoile, France), API ID 32C or API 20C AUX (BioMérieux), or MALDI Biotyper (Bruker Daltonics, Bremen, Germany). The institutions were either a university hospital or a teaching hospital: Nagasaki University Hospital, Aichi Medical University Hospital, Hyogo College of Medicine Hospital, Toyama University Hospital, Toranomon Hospital, Sasebo City General Hospital, and Sasebo Kyosai Hospital. For all isolates, nucleotides of ITS regions were sequenced as described previously (9) and analyzed using the ClustalW algorithm in MacVector software (version 14.0.3, MacVector, Inc., NC, USA). PCR/RFLP of IGS was performed to distinguish *C. guilliermondii*, *C. fermentati*, *C. carpophila*, *and C. xestobii* as previously described (10). Identification results are shown in Table 1. Among 39 *C. guilliermondii* clinical isolates, 38 isolates were confirmed as *C. guilliermondii* and one isolate as *Kodamaea ohmeri*. Conversely, among seven clinical isolates originally identified as *C. famata* by the VITEK 2 system, six isolates were re-identified as *Candida parapsilosis* and one isolate as *C. guilliermondii* complex; no true *C. famata* strain was

identified. The results of our study, in conjunction with previous reports (7, 8, 11, 12), suggest that *C. famata* is far less common as a cause of invasive candidiasis. The MALDI Biotyper misidentified *K. ohmeri*, as this species was not included in the database when the isolate was identified in 2013 (13). All three laboratory identification systems identified the correct *C. guilliermondii* complex clade, however, these systems are not able to accurately identify to a species level among the *C. guilliermondii* complex. The ITS sequences of the clinical *C. guilliermondii* and *C. fermentati* isolates matched those of the reference strains CBS566 and CBS2022, respectively, with 99.6–100% similarities. Clinical *C. fermentati* isolates differed from *C. guilliermondii* isolates by 3–5 bp in the ITS region sequences. The homology of nucleotide sequences of the ITS regions between these two species was 99.0–99.3%. *C. fermentati* (CBS2022) differed from *C. carpophila* (CBS5256) and *C. xestobii* (CBS5975) by two nucleotides. The PCR/RFLP analyses of the IGS regions confirmed that 27 isolates were *C. guilliermondii* and 12 isolates were *C. fermentati*. There were no *C. carpophila* or *C. xestobii* isolates.

Clinical information including specimen types, comorbidity, β-D glucan values measured by Fungitec G Test MK Ⅱ "Nissui" (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan; cutoff value: 20.0 pg/ml), initial antifungal agents administered, and therapeutic outcomes was reviewed retrospectively, for all 39 patients from which *C. guilliermondii*

87 complex had been isolated. The investigators determined whether isolates caused
88 infection or colonized based on clinical courses. The study protocol was approved by the
89 ethical review boards in all institutions that participated in this study. The registration
90 number of this study is 14122267 in the principal investigator institution Nagasaki
91 University Hospital. Patient characteristics are shown in Table 2. All statistical analyses
92 were carried out using Prism 6.0 (Graphpad software, Inc., 2012). Nominal variables were
93 compared using Fischer's exact test and continuous variables of patient characteristics
94 were compared using the Mann-Whitney U test. Of the 39 patients, 28 patients (71.8%)
95 were diagnosed with complicated malignancies, notably 17 patients (43.6%) had
96 underlying hematological cancer. The *C. guilliermondii* complex isolates were obtained
97 from 31 patients (79.5%) with a central venous catheter; 19 patients (48.7%) administered
98 with steroids; and 21 patients (53.8%) receiving antifungal therapy with micafungin (n =
99 14), itraconazole (n = 1), fluconazole (n = 2), voriconazole (n = 2), and liposomal
100 amphotericin B (n = 5). Among these patient characteristics, no significant difference was
101 found between the *C. guilliermondii* and *C. fermentati* groups. The only clinical
102 difference was that none of the 12 *C. fermentati* isolates were obtained from the
103 bloodstream whereas 81.5% (n = 22/27) of the *C. guilliermondii* isolates were obtained
104 from the bloodstream ($P < 0.0001$). The other *C. guilliermondii* isolates were from

105 cerebrospinal fluid (*n* = 1), ear discharge (*n* = 1), sputum (*n* = 2), and the urinary tract (*n*
106 = 1). In contrast, the majority of *C. fermentati* isolates were obtained from non-sterile
107 sites including stool (*n* = 7), sputum (*n* = 2), bile (*n* = 2), and the urinary tract (*n* = 1). In
108 previous studies, among the clinical cases diagnosed as *C. guilliermondii* infection, 77-
109 95% cases were actually caused by *C. guilliermondii* while 5-23% cases were due to *C.*
110 *fermentati*. (4, 5, 14, 15). The results of our study were in agreement with the previous
111 report that *C. guilliermondii* was more commonly isolated from the bloodstream than *C.*
112 *fermentati* (5). We collected the *C. guilliermondii* complex isolates from any type of
113 specimen regardless of whether they caused infections, in contrast, most previous studies
114 analyzed only infectious cases including candidemia. The elevation of serum β-D glucan
115 level was more frequently found in the *C. guilliermondii* group (*n* = 16, 64.0%) compared
116 to the *C. fermentati* group (*n* = 2, 16.7%) (*P* = 0.01). The findings in our study suggest
117 that *C. fermentati* colonizes at non-sterile sites but hardly invade into the bloodstream,
118 which may also be supported by the significantly low serum β-D glucan levels in the *C.*
119 *fermentati* group.

120 Antifungal susceptibility tests was performed using Sensititre YeastOne (SYO)
121 microtiter panel (TREK Diagnostic Systems, Ltd., East Grinstead, UK) (16). The MICs
122 of *C. guilliermondii* complex isolates were interpreted by the species-specific clinical

123 breakpoints (CBPs) (17) and the epidemiological cutoff values (ECVs) (18, 19). The
124 antifungal susceptibilities of the 27 *C. guilliermondii* and 12 *C. fermentati* isolates are
125 shown in Table 3. All the *C. guilliermondii* and *C. fermentati* isolates were susceptible to
126 micafungin, caspofungin, and anidulafungin, except one *C. guilliermondii* isolate that was
127 categorized as intermediate to anidulafungin when interpreted using the CBPs. All the *C.*
128 *guilliermondii* and *C. fermentati* isolates were categorized as wild-type for echinocandins,
129 amphotericin B, and 5-flucytosine by using the ECVs. *C. guilliermondii* is known to show
130 intrinsically higher echinocandin MIC values than other *Candida* species (2), and a recent
131 study has reported that 9.1–27.2% of *C. guilliermondii* isolates were not susceptible to
132 echinocandins (6). In contrast, most *C. guilliermondii* isolates were susceptible to
133 echinocandins in this study. The reason for this difference is unclear but may be related
134 to geographic location, prior exposure to echinocandins, or other unknown factors.
135 Nonetheless, all the *C. fermentati* isolates were susceptible to the nine antifungal agents
136 tested in this study, in agreement with the previous reports from China and Taiwan (4, 5).
137 Taken together, these findings suggest a lower frequency of resistant strains in *C.*
138 *fermentati* and certain variations in the frequency of echinocandin resistance in *C.*
139 *guilliermondii*. According to previous studies, approximately 5–15% of *C. guilliermondii*
140 isolates are azole resistant (2, 4, 5, 15). Correspondingly, 7.4–14.8% of *C. guilliermondii*

141 isolates were categorized as non-wildtype for azoles according to the ECVs in our study.
142 All of the *C. fermentati* isolates were categorized as wild-type for all azoles tested in this
143 study.

144 The sequences of the *FKS1* hot spot regions of four *C. guilliermondii* and eight
145 *C. fermentati* isolates were analyzed. Hot spot regions were amplified with the following
146 forward and reverse primer pairs: HS1-F (5′-AATGGGCTGGTGCTCAACAT-3′) and
147 HS1-R (5′-CCTTCAATTTCAGATGGAACTTGATG-3′) for hot spot 1, and HS2-F (5′-
148 AAGATTGGTGCTGGTATGGG-3′) and HS2-R (5′-GTGGCGAAACCTCTACCAGT-
149 3′) for hot spot 2. The reference sequences of *FKS1* were retrieved from the NCBI
150 database and sequence analyses were performed with MacVector software. Decreased
151 echinocandin susceptibility of *C. guilliermondii* was attributed to the intrinsic amino acid
152 changes in the hot spot 1 region of Fks1 (20-22). However, to our knowledge, there is
153 only one study that analyzed the DNA sequence of the *FKS1* hot spot region for a *C.*
154 *fermentati* clinical isolate (23). The present study revealed that all the *C. guilliermondii*
155 and *C. fermentati* isolates harbored two polymorphisms (L633M and T634A) in the first
156 Fks1 hot spot, which may account for intrinsically higher echinocandin MICs of these
157 strains. No mutation was found in the second Fks1 hot spot region.

158 To examine in vivo echinocandin susceptibility of *C. guilliermondii*, mice were

159 infected intravenously with the *C. guilliermondii* clinical isolates (NGSCG1 and
160 ACHCG213) and treated with micafungin. The *Candida glabrata* wild-type strain
161 CBS138 (24) was used as a control as micafungin is known to be highly active against *C.*
162 *glabrata*. In vitro MICs of micafungin were 1 μg/ml for NGSCG1, 0.25 μg/ml for
163 ACHCG213, and 0.03 μg/ml for CBS138. All animal experiments were performed in full
164 compliance with the Guide for the Care and Use of Laboratory Animals (National
165 Research Council, National Academy Press, Washington DC, 2011) and the institutional
166 regulations and guidelines for animal experimentation after pertinent review and approval
167 by the Institutional Animal Care and Use Committee of Nagasaki University under
168 protocol number 1407281164. Specific-pathogen-free, 7-week-old female BALB/c mice
169 (Japan SLC Inc., Shizuoka, Japan) were rendered neutropenic by intraperitoneal
170 administration of cyclophosphamide (Sigma-Aldrich, St; Louis, MO, USA) 4 days before
171 infection (150 mg/kg), 1 day before infection (100 mg/kg), 2 and 5 days post infection
172 (100 mg/kg) (25). The mice were infected intravenously through the lateral vein with 0.2
173 ml of the *Candida* cell suspension. The actual CFUs in the inocula were confirmed by
174 plating serial dilutions of cell suspension on YPD plates and were as follows: 7.2×10^6
175 cells for NGSCG1, 8.6×10^6 cells for ACHCG213, and 1.6×10^6 cells for CBS138.
176 Micafungin (Astellas Pharma Inc., Tokyo, Japan) was administered at 4 mg/kg/day

177 intraperitoneally for seven consecutive days in a 0.2 ml volume commencing 2 h post
178 infection. Taking into consideration the previous AUC data of micafungin in mice (26,
179 27), 4 mg/kg/day intraperitoneally administration to mice is expected to correspond to
180 approximately 100 mg/day administration to humans. The mice were euthanized 7 days
181 after infection. No mice died before euthanasia in this experiment. Appropriate dilutions
182 of organ homogenates were plated on YPD agar and the CFUs per organ were calculated.
183 Differences of fungal burden between the treatment and control groups were examined
184 using the Mann-Whitney test. In mice infected with *C. guilliermondii* isolates NGSCG1
185 and ACHCG213, micafungin at 4 mg/kg was effective at reducing the liver fungal burden
186 against the controls ($P = 0.0002$ for NGSCG1 and $P = 0.0006$ for ACHCG213). However,
187 the overall CFU reduction for *C. guilliermondii* was clearly less than that for *C. glabrata*
188 CBS138 (Figure 1). In mouse kidneys infected with *C. guilliermondii*, micafungin was
189 not effective to reduce fungal burden compared to the non-treatment controls ($P = 0.98$
190 for NGSCG1 and $P = 0.38$ for ACHCG213), although it significantly reduced the CFUs
191 in the liver and kidneys of mice infected with *C. glabrata* CBS138 ($P = 0.0002$). The
192 lower efficacy of micafungin against *C. guilliermondii* may be due to higher echinocandin
193 MIC values than in *C. glabrata*. A 4 mg/kg dose of micafungin was effective at reducing
194 the liver fungal burden in mice infected with *C. guilliermondii* isolates, yet ineffective in

195 the kidneys. It has been reported that micafungin concentrations in kidney tissues are less
196 than those in liver tissues (28), and micafungin translocates rapidly in and out of the
197 kidneys (29). The differences in micafungin concentrations in the kidney and liver tissues
198 may be reflected in therapeutic efficacy at those organs, although we were not able to
199 measure the actual drug concentrations in our mouse experiments. Nevertheless, our
200 study showed that, at a clinical dose, micafungin had a poor efficacy against *C.*
201 *guilliermondii*, while it was markedly more effective for *C. glabrata*.

202 To examine differences in phenotype between *C. guilliermondii* and *C.*
203 *fermentati,* we performed various assays related to pathogenicity. Biofilm formation
204 capacity of *Candida* cells was examined using the 2,3-bis(2-methoxy-4-nitro-5-sulfo-
205 phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay (30). There was no
206 significant difference in the capacity of biofilm formation between *C. guilliermondii*
207 (optical density at 492 nm: 0.112 ± 0.027) and *C. fermentati* (0.081 ± 0.026) (Kruskal-
208 Wallis test, $P = 0.059$). Biofilm formation capacity of *C. guilliermondii* and *C. fermentati*
209 was significantly less than that of the *Candida albicans* wild-type strain SC5314 (0.774
210 ± 0.036), respectively ($P = 0.039$ for *C. guilliermondii* vs *C. albicans* and $P = 0.0001$ for
211 *C. fermentati* vs *C. albicans*). We also performed spot dilution tests as described
212 previously (31) and found that *C. fermentati* isolates were more sensitive to H_2O_2 than *C.*

213 *guilliermondii* isolates were (Figure 2A). There were no differences in sensitivity to other
214 oxidative stress inducers including diamide and menadione, osmotic stresses induced by
215 sorbitol and NaCl, acid and alkaline stresses (pH 2.2–9.4), and growth at 30°C and 37°C
216 between *C. guilliermondii* and *C. fermentati* strains tested in this study (data not shown).
217 To further examine H_2O_2 sensitivity of these species, viable cell counts of nine isolates
218 for each of *C. guilliermondii* and *C. fermentati* were evaluated after incubation with 5
219 mM H_2O_2 for 4 h. To avoid potential for bias, these isolates were selected by considering
220 medical institution, specimen type, and antifungal susceptibility profiles. Logarithmic-
221 phase cells were suspended in PBS (pH = 7.2) at the concentration of 5×10^6 cells/ml and
222 treated with H_2O_2 at 30°C with agitation (250 rpm). Serial dilutions of cell suspensions
223 were plated on YPD agar and incubated at 30°C for 48 h to count viable cells. H_2O_2
224 exerted significant fungicidal effects against *C. fermentati* (Figures 2B and C). The
225 average viable cell counts before treatment were 6.8 ± 0.3 -log cells/ml for *C.*
226 *guilliermondii* and 6.7 ± 0.3 -log cells/ml for *C. fermentati*. A 4-h treatment with 5 mM
227 H_2O_2 reduced viable cell counts of *C. guilliermondii* and *C. fermentati* by 1.1 ± 0.4 and
228 3.4 ± 0.5 -log cells/ml, respectively. There was a significant difference between the two
229 groups (Mann-Whitney test, $P < 0.001$). These results suggest that *C. fermentati* was more
230 sensitive to H_2O_2 than *C. guilliermondii.*

231 In the host environment, phagocytes are the first line of defense against fungal
232 infections. These cells produce reactive oxygen species such as superoxide, H_2O_2, and
233 hydroxyl radicals for damaging biomolecules and killing phagocytosed pathogens (32,
234 33). Murine macrophages are known to be capable of killing microbes including *Candida*
235 species (34, 35). We performed macrophage killing assays against *C. guilliermondii* and
236 *C. fermentati* using murine RAW 264 macrophages. RAW 264 cells were cultured at 37°C
237 in 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich)
238 supplemented with 10% fetal bovine serum (Life technologies, Japan) and 1% penicillin
239 and streptomycin (Sigma-Aldrich). Macrophages were scraped with trypsin-EDTA
240 (0.25%), phenol red (Invitrogen, Carlsbad, CA, USA) and rinsed in DMEM. The nine
241 clinical isolates of *C. guilliermondii* and *C. fermentati*, which were the same strains used
242 for the H_2O_2 assay, were incubated in YPD broth at 30°C and logarithmic-phase cells
243 were prepared. The killing assay was performed by reference to the methods described
244 previously (36, 37). On the basis of pilot studies, 8.0×10^4 macrophages were co-cultured
245 with 5.3×10^3 *Candida* cells (15:1 ratio) in 1.5 ml microtubes with rotation at 37°C for 4
246 h. The cultures were sonicated, diluted and spread on YPD agar to count the viable cells.
247 CFUs of the co-cultures were compared with CFUs of growth control tubes containing
248 *Candida* cells without macrophages. Percentage of killing ratio was calculated as [1 -

249 (CFUs from co-culture tubes / CFUs from control tubes)]. The cell ratio before treatment
250 were 14.7 ± 3.4: 1 (macrophage: *Candida*) for *C. guilliermondii* and 13.1 ± 5.5: 1 for *C.*
251 *fermentati*. Viable cell counts after 4-h treatment were 2.43 ± 0.80 × 10^4 cells/ml for *C.*
252 *guilliermondii* and 2.56 ± 1.11 × 10^4 cells/ml for *C. fermentati* without RAW 264 cells,
253 and 2.39 ± 0.81 × 10^4 cells/ml for *C. guilliermondii* and 1.86 ± 0.92 × 10^4 cells/ml for *C.*
254 *fermentati* with RAW 264 cells. The killing ratio of *C. fermentati* was significantly higher
255 than that of *C. guilliermondii*, at 24.4 ± 14.6 % versus -0.6 ± 14.4 %, respectively (Mann-
256 Whitney test, $P < 0.01$) (Figure 2D).
257 In conclusion, the present study demonstrates that *C. guilliermondii* and *C.*
258 *fermentati* are closely related but have different microbiological and clinical
259 characteristics. Among the *C. guilliermondii* complex, *C. guilliermondii* was highly
260 associated with bloodstream infections but *C. fermentati* was not. This may be explained,
261 at least in part, by the lower resistance of *C. fermentati* to oxidative stress and killing by
262 macrophages. Since no *C. guilliermondii* was accidentally identified as *C. fermentati* and
263 all of the *C. fermentati* isolates were more susceptible and less pathogenic, it may not be
264 needed to distinguish *C. fermentati* from *C. guilliermondii* in clinical practice. However,
265 in vitro echinocandin MICs for *C. guilliermondii* should be regarded with some caution,
266 because this species was less susceptible to micafungin in vivo. A limitation of this study

is the limited number of isolates analyzed, therefore our findings need to be confirmed in future studies.

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Table 1. Identification results by laboratory identification systems and ITS sequencing

Identification system	Species (no. isolates)	
	Original identification	Confirmation by ITS sequencing
VITEK 2 system	*C. guilliermondii* (6)	*C. guilliermondii* complex (6)
	C. famata (7)	*C. guilliermondii* complex (1) *C. parapsilosis* (6)
API series	*C. guilliermondii* (18)	*C. guilliermondii* complex (18)
MALDI Biotyper system	*C. guilliermondii* (15)	*C. guilliermondii* complex (14) *Kodamaea ohmeri* (1)

ITS, internal transcribed spacer

434 **Table 2.** Patient characteristics associated with *C. guilliermondii* complex isolates

Characteristic	*C. guilliermondii* (*n* = 27)	*C. fermentati* (*n* = 12)	*P* value
Age (yr), mean ± SD	63.3 ± 18.7	61.2 ± 19.0	0.75
Male sex, no. (%)	20 (74.1)	7 (58.3)	0.46
Co-malignancies, no. (%)	18 (66.7)	10 (83.3)	0.45
Steroid use, no. (%)	11 (40.7)	8 (66.7)	0.18
Central venous catheter, no. (%)	22 (81.5)	9 (75.0)	0.68
In-hospital death, no. (%)	13 (48.1)	6 (50.0)	1.00
Antifungal therapy, no. (%)	12 (44.4)	9 (75.0)	0.10
Elevated β-D glucan, no. (%)	16 (64.0) [a]	2 (16.7)	0.01
Bloodstream isolates, no. (%)	22 (81.5)	0 (0)	< 0.0001
Cause of infection, no. (%)	24 (88.9)	0 (0)	< 0.0001

435 [a] Serum β-D glucan was measured for 25 patients.

436

Table 3. In vitro susceptibility of 27 *C. guilliermondii* isolates and 12 *C. fermentati* isolates to nine antifungal agents as interpreted by breakpoints for echinocandins and epidemiological cutoff values for other agents

***C. guilliermondii* complex**	**MIC (µg/ml)**			**No. (%) of isolates by ECVs**		**No. (%) of isolates by CBPs**		
	Range	MIC_{50}	MIC_{90}	WT	Non-WT	S	I	R
***C. guilliermondii* (*n* = 27)**								
Fluconazole	2 – >256	4	128	23 (85.2)	4 (14.8)			
Itraconazole	0.25 – >16	0.5	2	24 (88.9)	3 (11.1)			
Voriconazole	0.03 – 8	0.12	0.5	25 (92.6)	2 (7.4)			
Posaconazole	0.06 - 2	0.25	0.5	25 (92.6)	2 (7.4)			

5-Flucytosine	≤0.06	≤0.06	≤0.06	27 (100)	0 (0)			
Micafungin	0.25 – 1	0.5	1	27 (100)	0 (0)	27 (100)	0 (0)	0 (0)
Caspofungin	0.12 - 1	0.5	1	27 (100)	0 (0)	27 (100)	0 (0)	0 (0)
Anidulafungin	0.5 - 4	2	2	27 (100)	0 (0)	26 (96.3)	1 (3.7)	0 (0)
Amphotericin B	0.25 – 2	0.5	1	27 (100)	0 (0)			
C. fermentati								
(*n* = 12)								
Fluconazole	2 – 8	4	4	12 (100)	0 (0)			
Itraconazole	0.25 – 0.5	0.25	0.5	12 (100)	0 (0)			
Voriconazole	0.06 – 0.25	0.12	0.25	12 (100)	0 (0)			

Posaconazole	0.03 – 0.25	0.12	0.25	12 (100)	0 (0)			
5-Flucytosine	≤0.06 – 0.12	≤0.06	0.12	12 (100)	0 (0)			
Micafungin	0.25 – 1	0.5	1	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Caspofungin	0.25 - 1	0.5	0.5	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Anidulafungin	0.5 - 2	1	2	12 (100)	0 (0)	12 (100)	0 (0)	0 (0)
Amphotericin B	0.5 – 1	0.5	1	12 (100)	0 (0)			

Epidemiological cutoff values (ECVs) (42): fluconazole 8 μg/ml, itraconazole 1 μg/ml, voriconazole 0.25 μg/ml, posaconazole 0.5 μg/ml, 5-flucytosine 1 μg/ml, micafungin 2 μg/ml, caspofungin 2 μg/ml, anidulafungin 4 μg/ml, and amphotericin B 2 μg/ml.

Species-specific clinical breakpoints (CBPs) of echinocandins in CLSI M27-S4 (16): S, ≤ 2 μg/ml; I, 4 μg/ml; R, ≥ 8 μg/ml.

Figures and Figure legends

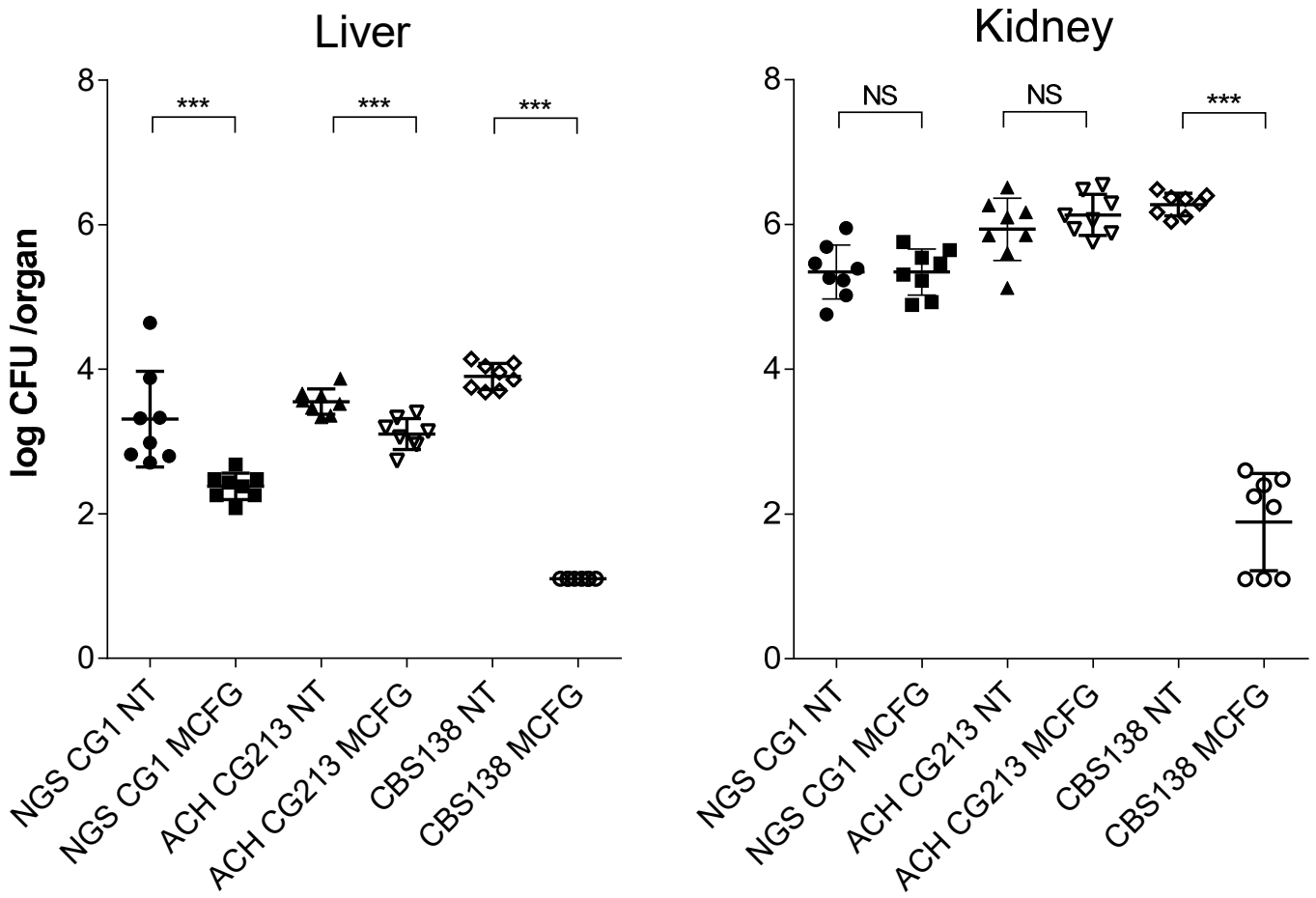
Figure 1. Treatment efficacy of micafungin against *C. guilliermondii* clinical isolates and a *C. glabrata* wild-type strain in a mouse model of disseminated candidiasis

Immunocompromised mice were inoculated with *C. guilliermondii* NGSCG1, *C. guilliermondii* ACHCG213, and *C. glabrata* CBS138. Micafungin was administered at 4 mg/kg/day intraperitoneally for seven consecutive days. The number of cells recovered from the liver and bilateral kidneys is indicated for individual mice in the plots and error bars represent SDs. NT indicates no treatment. Asterisks and NS indicate statistically significant differences (*** $P < 0.001$) and no significance ($P > 0.05$), respectively.

Figure 2. Growth and killing assay in the presence of H_2O_2 and killing assay by RAW 264

(A) Serial dilutions of logarithmic-phase cells of *C. guilliermondii* and *C. fermentati* strains were spotted onto SC plates containing H_2O_2 at the indicated concentrations. *C. guilliermondii* strains: NGSCG3, TYMCG251, and CBS566; *C. fermentati* strains: NGSCF1, ACHCF243, and CBS2022. (B) Logarithmic-phase cells of *C. guilliermondii* and *C. fermentati* were treated with 5 mM H_2O_2 for 4 h. The number of cells at 0 h; *C. guilliermondii* was 6.8 ± 0.3 -log cells/ml and *C. fermentati* was 6.7 ± 0.3 -log cells/ml.

After 4 h treatment; *C. guilliermondii* was 5.7 ± 0.6 -log cells/ml and *C. fermentati* was 3.3 ± 0.5 -log cells/ml. (C) The reduced viable cell counts of *C. guilliermondii* and *C. fermentati* were 1.1 ± 0.4 and 3.4 ± 0.5 -log cells/ml, respectively. There was a significant difference between the two groups (*** $P < 0.001$). (D) Logarithmic-phase cultures of *Candida* cell suspensions were co-cultured with RAW 264 cells at 37°C for 4 h. Mean in vitro killing of *C. guilliermondii* and *C. fermentati* is expressed as the percent reduction of CFUs recovered from co-cultures compared with CFUs from control cultures (*Candida* cells without macrophages). Dots represent results of the killing ratio of individual clinical isolates. The percent killing ratios of *C. guilliermondii* and *C. fermentati* were -0.6 ± 14.4 % and 24.4 ± 14.6 %, respectively. There was a significant difference between the two groups (**$P < 0.01$).



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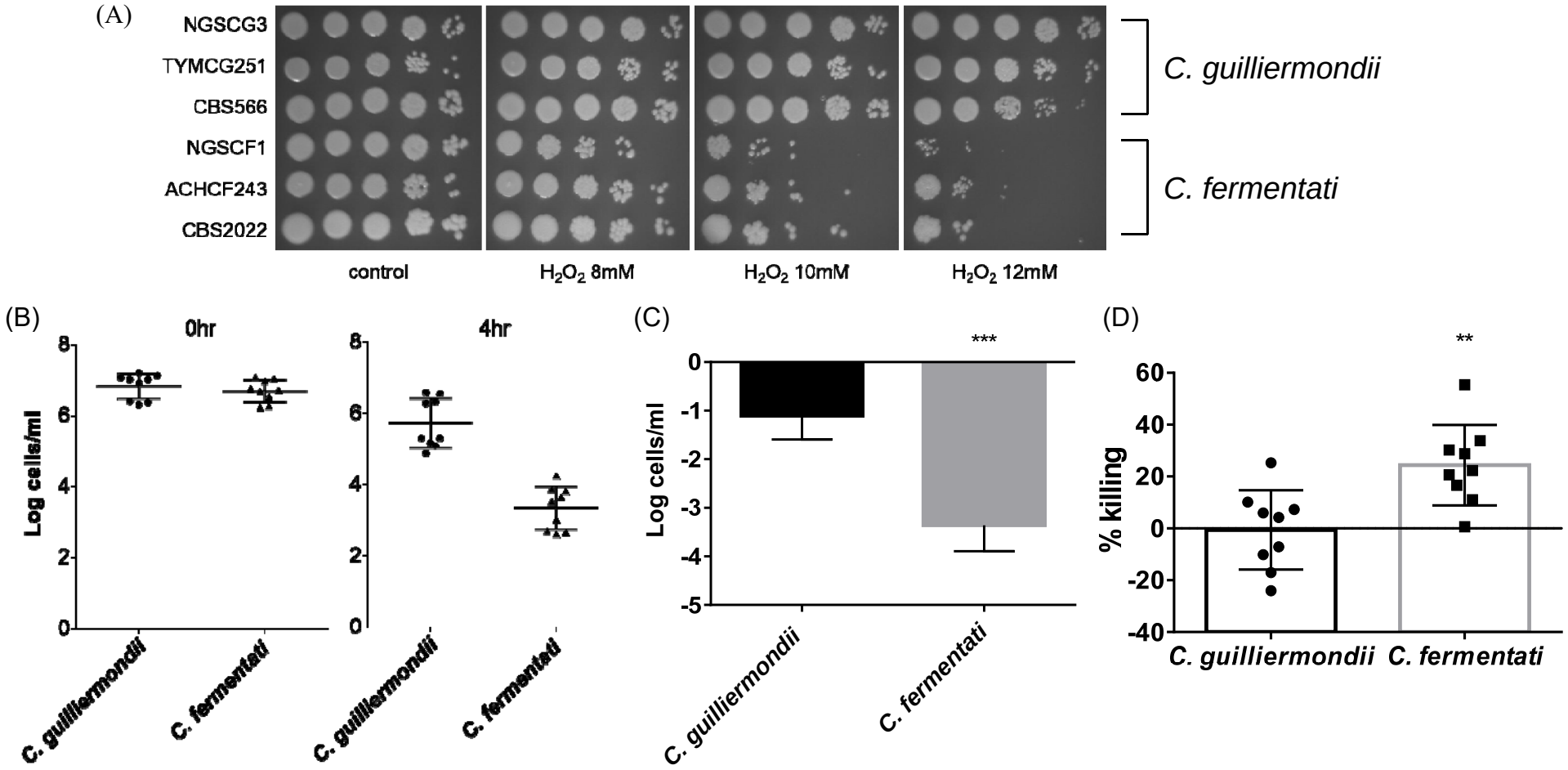

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