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1 **Impact of the physiological state of fungal spores on their inactivation by active chlorine**  
2 **and hydrogen peroxide**

3

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15

16 **Keywords:** *Aspergillus*, *Cladosporium*, *Mucor*, *Penicillium*, fungicide, disinfectant, predictive  
17 **mycology**

18

19

20 **Abstract**

21 This study aimed at assessing the impact of the physiological state of fungal spores on  
22 inactivation by sodium hypochlorite, 0.1% and 0.2% active chlorine, and 3% hydrogen  
23 peroxide. In this context, two physiological states were compared for 4 fungal species (5  
24 strains). The first physiological state corresponded to fungal spores produced at 0.99  $a_w$  and  
25 harvested using an aqueous solution (laboratory conditions), while the second one  
26 corresponded to fungal spores produced under a moderate water stress (0.95  $a_w$ ) and dry-  
27 harvested (mechanical harvesting without use of any water, mimicking food plant conditions).  
28 *Aspergillus flavus* “food plant” conidia were more resistant to all tested fungicide molecules  
29 than the “laboratory” ones. The same phenomenon was observed for *Penicillium commune*  
30 UBOCC-A-116003 conidia treated with hydrogen peroxide. However, this isolate did not  
31 exhibit any inactivation difference between “laboratory” and “food plant” conidia treated with  
32 sodium hypochlorite. Similarly, the physiological state of *Cladosporium cladosporioides*  
33 conidia did not impact the efficacy of the tested biocides. *P. commune* UBOCC-A-112059  
34 “food plant” and “laboratory” conidia were more resistant to hydrogen peroxide and sodium  
35 hypochlorite, respectively. As for *Mucor circinelloides*, “laboratory” spores were more  
36 resistant to all disinfectant than the “food plant” ones. Noteworthy, regardless of the  
37 physiological state, all *M. circinelloides* and *C. cladosporioides* conidia were inactivated for 5  
38 min treatment at 0.2% active chlorine and for 2.5 min treatment at 0.1% active chlorine, while  
39 the conidia of all the other species remained viable for these treatments. The obtained data  
40 indicate that the efficacy of disinfectant molecules depends not only on the encountered  
41 fungal species and its intraspecific diversity but also on the spore physiological state.

42

## 44 1. Introduction

45 Efficacy of fungicide molecules depends on various treatment conditions, i.e., nature of the  
46 disinfectant, contact time, concentration, application conditions (temperature, pH, relative  
47 humidity, dirtiness of the surfaces, presence of surfactants...) and mode (e.g. fumigation,  
48 liquid application) and obviously the targeted fungi. For sanitization efficacy tests, the  
49 standard fungal strains are *Aspergillus brasiliensis* (ATCC 16404) and *Candida albicans*  
50 (ATCC 10231). According to the European Committee for Standardization, other species can  
51 be tested, i.e., *Absidia corymbifera* (IP 1129 75), *Candida albicans* (IP 1180 79),  
52 *Cladosporium cladosporioides* (IP 1232 80), *Penicillium verrucosum* var. *cyclopium* (IP 1231  
53 80) to assess fungicidal efficacy (KSG France, 2021). Beyond the fact that the proposed  
54 species may not be the most representative of food plant contaminants and, as recently shown  
55 by Scaramuzza et al. (2020), might even be more sensitive than the latter; in all cases, fungal  
56 spores have to be produced under optimal growth conditions (i.e., temperature, time,  
57 atmosphere, medium, (AFNOR NF T 72-281, 2014; European Standard 1275, 2006;  
58 European Standard 1650, 2019; European Standard 13624, 2013; European Standard  
59 13697+A1, 2019). These standards recommend Malt Extract Agar (pH 5.6, 0.99  $a_w$ ) to  
60 produce fungal spores that are subsequently diluted into peptonized water. In contrast to these  
61 spores produced in laboratory conditions, in food plant conditions, spores are airborne and not  
62 rehydrated, and unlikely to be produced under optimal conditions.

63 The physiological state of *Penicillium* spp. strains was affected by temperature, water activity  
64 and pH during conidia production (Nguyen van Long et al., 2017a). Moreover, the impact of  
65 the harvesting protocol on the susceptibility to disinfectants can be important as, for example,  
66 wet harvested *Rhizopus stolonifer* conidia had a different physiological state than dry-  
67 harvested ones. Even brief exposure (30 min) to water during harvesting caused spores to

68 engage metabolic activity (Nicherson et al., 1981). The effects of environmental conditions  
69 during sporogenesis on the germination time is well documented (Blaszyk et al., 1998; Judet  
70 et al., 2008; Nanguy et al., 2010; Nguyen van Long et al., 2017a, 2017b; Lattab et al., 2012;  
71 Ruijten et al., 2020; Stevenson et al., 2016). Less information on the impact of environmental  
72 conditions on the susceptibility to disinfectants is available. Dry-harvested *Penicillium*  
73 conidia exposed to ethanol vapours for 24h did not show any inactivation whereas aqueous  
74 suspensions of *P. digitatum* and *P. italicum* conidia exhibited about 3 log and 2 log  
75 inactivation, respectively (Dao and Dantigny, 2009). The same authors showed that the  
76 contact time with ethanol vapours (0.67 kPa) required to inactivate 4 log *P. chrysogenum* and  
77 *P. digitatum* conidia produced at either 0.99  $a_w$  or in the 0.85-0.90  $a_w$  range were about 30h  
78 and 120h, respectively (Dao and Dantigny, 2009).

79 Production of conidia at reduced water activity increases the amount of protective compatible  
80 solutes in the cytoplasm (Andersen et al., 2006; Chandler et al., 1984; de Lima Alves et al.,  
81 2015; Hallsworth and Magan, 1995; Magan, 2006, 2007; Nesci et al., 2014; Rangel et al.,  
82 2015; Wyatt et al., 2013, 2015) that can contribute to a certain resistance. It was also  
83 hypothesized that both the production of conidia at reduced water activity and application of a  
84 dry-harvesting protocol contributed to lower the intracellular water activity of fungal spores  
85 (Dao and Dantigny, 2009), thus explaining their resistance to ethanol which is an hydrophilic  
86 molecule. This hypothesis was strengthened by the observation that for 1 min exposure to 70%  
87 ethanol, only dry-harvested conidia of four *Penicillium commune* strains produced at 0.95  $a_w$   
88 exhibited survivors, while suspensions of conidia produced at 0.995  $a_w$  were completely  
89 inactivated (Visconti et al. 2020).

90 This study aimed at extending the comparison between “laboratory” conidia (production at  
91 0.995  $a_w$  and harvested using an aqueous solution) and “food plant” (production at 0.95  $a_w$   
92 and dry-harvested) conidia to other fungal species and disinfectant molecules. The ethanol

93 resistant *P. commune* UBOCC-A-116003 and the ethanol sensitive *P. commune* UBOCC-A-  
94 112059 (Visconti et al. 2020) were selected, in addition to species that are frequently isolated  
95 from dairy product environments, namely *Cladosporium cladosporioides*, *Mucor*  
96 *circinelloides* and *Aspergillus flavus*. The tested disinfectant molecules corresponded to  
97 sodium hypochlorite (0.1 and 0.2% active chlorine) and hydrogen peroxide (3%).

98

## 99 **2. Material and methods**

### 100 *2.1. Molds*

101 The studied molds were provided by the Université de Bretagne Occidentale Culture  
102 Collection (UBOCC, <https://www.univ-brest.fr/ubocc/>) and were originally isolated from  
103 spoiled dairy products (Table 1). Molds were maintained on Potato Dextrose Agar (PDA)  
104 medium at 4°C.

105

### 106 *2.2. Spores production*

107 “Laboratory” spores were produced from mycelium grown on PDA at 0.995  $a_w$  for 7 days at  
108 25°C and harvested in sterile saline solution (NaCl, 9 g/l of water) containing Tween 80  
109 (0.015% v/v).

110 “Food plant” spores were produced from mycelium grown on PDA at 0.950  $a_w$  for 7 days at  
111 25°C. Water activity was adjusted by substituting a part of the water with an equal weight of  
112 glycerol. The relative amount of glycerol was 20.6% (w/w) to obtain PDA media at 0.950  $a_w$ .

113 Spores were harvested mechanically without contact with any liquid solution (dry-harvest)  
114 according to Dao and Dantigny (2009). Briefly, ten sterile glass beads (diameter 3 mm) were  
115 deposited on the mycelium and the Petri dish was shaken gently to detach the conidia. Then,  
116 the plate was turned upside-down and the conidia were harvested on the lid by gently tapping  
117 100 times on the bottom of the Petri dish.

118

119 *2.3. Disinfectants*

120 Sodium hypochlorite test solutions were prepared from a stock solution diluted in distilled  
121 water containing Tween 80 (0.015%, v/v). The stock solution was prepared from 250 ml  
122 bleach boxes (Eau de Javel La Croix, Bois-Colombes, France) that contained 4.8% active  
123 chlorine (5% hypochlorite), diluted with distilled water according to the supplier's  
124 recommendation to reach final concentrations of 0.1 and 0.2% active chlorine. The co-  
125 formulants mentioned by the supplier were 5-15% chlorine bleach and sodium hydroxide. The  
126 hydrogen peroxide test solution was prepared from 30% hydrogen peroxide solution (Sigma  
127 Aldrich, Saint-Quentin Fallavier, France) diluted in distilled water containing Tween 80  
128 (0.015%, v/v) to reach a 3% final concentration.

129

130 *2.4. Disinfectant testing procedures*

131 Treatment of “food plant” spores started by applying 10 ml of biocide solution to the dry-  
132 conidia on the lid. For *M. circinelloides*, this volume was reduced to 5 ml as fewer spores  
133 were harvested. Prior to the first sampling, the suspension (biocide + conidia) was transferred  
134 to a Falcon tube and homogenized by vortexing for 10 s. Treatment of “laboratory” spores  
135 began when 1ml of spore suspension was transferred in 9 ml of biocide solution and the mix  
136 vortexed. For sodium hypochlorite, spores survival was measured every 2.5 min for 20 min  
137 except for *M. circinelloides* for which an additional contact time of 1 min was done. For  
138 hydrogen peroxide, spores survival was measured after 1, 5, 10, 15, 30 and 60 min of contact  
139 time. Both biocides were neutralized by diluting ten-fold the suspension in 1% sodium  
140 thiosulfate pentahydrate diluted in saline aqueous solution (NaCl, 9 g/l) containing Tween 80  
141 (0.015% v/v). The initial amount of “food plant” spores ( $N_0$ ) was evaluated by counting on a  
142 Malassez cell according to Dao et al. (2008). The method was based on the principle that the

143 application of biocide does not affect the morphology of the spores during the time of the  
144 experiment so it is possible to count under the microscope all the spores treated including  
145 inactivated ones.

146

#### 147 2.5. Viability assessment

148 After neutralization, the treated spores were grown on PDA at 25 °C for 3 days by spreading  
149 100 µl of sample and four subsequent decimal dilutions. *M. circinelloides* spores were grown  
150 only for 1 day. Only counts (N) in the range of 10 to 100 colonies per plate were considered.  
151 Inactivation of conidia was expressed as the logarithmic reduction factor,  $\log_{10} (N/N_0)$ .

152

### 153 3. Results

#### 154 3.1. Impact of disinfectant molecules on “laboratory” spores

155 At 0.1% active chlorine, *C. cladosporioides* was the most sensitive species (Fig. 1A), as for a  
156 contact time of 2.5 min, *C. cladosporioides* showed 2.8 log inactivation. Inactivation were  
157 less than or equal to 0.2 log for all the other tested species. At 12.5 min of exposure, *P.*  
158 *commune* only exhibited survivors, the UBOCC-A-112059 isolate was more resistant than the  
159 UBOCC-A-116003.

160 For a contact time of 2.5 min to 0.2% active chlorine, total inactivation of *C. cladosporioides*  
161 and *M. circinelloides* spores was observed, (Fig. 1B). At 7.5 min of exposure, *P. commune*  
162 only exhibited survivors. Inactivation values were 1.9 log and 3.1 log for the UBOCC-A-  
163 112059 and UBOCC-A-116003 strains, respectively. Under these experimental conditions, all  
164 *A. flavus* conidia were inactivated.

165 By increasing the active chlorine concentration from 0.1 to 0.2%, the contact time leading to a  
166 complete inactivation of the conidia was reduced from 5 to 2.5 min and, from 15 to 10 min,



167 for *C. cladosporioides* and *P. commune*, respectively. Regardless of the tested concentration,  
168 the former and the latter species were the most sensitive and most resistant to active chlorine,  
169 respectively.

170 In contrast to active chlorine, *P. commune* was the most sensitive species to 3% hydrogen  
171 peroxide (Fig. 1C). For a 10 min contact time, all spores of the two tested strains were  
172 inactivated. After 30 min of exposure, all *A. flavus* spores were inactivated, inactivation being  
173 greater than 4 log. In contrast to this species, inactivation was only 0.2 log for *C.*  
174 *cladosporioides* and *M. circinelloides*, which were the most resistant species.

175

### 176 3.2. Impact of fungicide molecules on "food plant" spores

177 As for the physiological "food plant" state, *M. circinelloides* was the most susceptible mold to  
178 0.1% active chlorine. At 2.5 min of exposure, all *M. circinelloides* spores were inactivated,  
179 inactivation being greater than 4.3 log (Fig. 2A). For this contact time, the inactivation of *C.*  
180 *cladosporioides* was equal to 2.7 log, and less than or equal to 0.2 log for *A. flavus* and *P.*  
181 *commune*. For the "food plant" physiological state, *A. flavus* was the most resistant species,  
182 the only one to exhibit survivors for a contact time of 17.5 min.

183 By increasing the active chlorine concentration, the contact time necessary to inactivate all the  
184 spores was reduced. After 2.5 min of exposure, all *C. cladosporioides* conidia and *M.*  
185 *circinelloides* spores were inactivated, while inactivation was lower than or equal to 0.4 log  
186 for *A. flavus* and *P. commune* (Fig. 2B). At 0.2% active chlorine, *P. commune* UBOCC-A-  
187 116003 was more resistant than the UBOCC-A-112059 strain. No *A. flavus* survivors were  
188 observed for a contact time of 12.5 min.

189 *A. flavus* was the most sensitive species to 3% hydrogen peroxide. A complete inactivation  
190 was observed for a contact time of 30 min (Fig. 2C). After 60 min exposure, only *C.*

191 *cladosporioides* (1.2 log inactivation) and *P. commune* UBOCC-A-112059 (2.4 log  
192 inactivation) exhibited survivors. All spores of *M. circirnelloides* and *P. commune* UBOCC-A-  
193 116003 were inactivated (> 4 and > 4.4 log, respectively).

194

### 195 3.3. Impact of the physiological state on the disinfectant efficacy

196 In order to assess the impact of the physiological state on the efficacy of the fungicide  
197 molecules, inactivation were compared at the same time, although this time depended on the  
198 considered molecule and species. Whenever possible, the shortest contact time leading to no  
199 survivors, for either “laboratory” or “food plant” spores, was chosen (Table 2). Four different  
200 cases were distinguished, i) “laboratory” spores were more resistant than “food plant” ones, ii)  
201 no significant differences were observed between the two physiological states, iii)  
202 impossibility to determine whether the two kind of spores exhibited significant differences  
203 and iv) “food plant” spores were more resistant than “laboratory” ones.

204 For *M. circirnelloides*, the "laboratory" spores were more resistant than the "food plant"  
205 spores to two disinfectants. For a contact time of 2.5 min at 0.1% active chlorine, inactivation  
206 was 0.1 log and greater than 4.3 log for the "laboratory" and "food plant" spores, respectively.  
207 The difference in inactivation between the two physiological states was greater than 4.2 log.  
208 After exposure to 0.2% active chlorine for 1 min, inactivation was 0.2 log and greater than 4.3  
209 log for "laboratory" and "food plant" spores, respectively. The difference in inactivation  
210 between the two physiological states was greater than 4.1 log. For *P. commune* UBOCC-A-  
211 112059, the "laboratory" conidia were more resistant than the "food plant" ones to active  
212 chlorine. For a contact time of 12.5 min at 0.1% active chlorine, inactivation was 1.5 log and  
213 3 log for the "laboratory" and "food plant" conidia, respectively. The difference in  
214 inactivation between the two physiological states was 1.5 log. At 0.2% active chlorine for 1

215 min, inactivation was 1.9 and 4.6 log for "laboratory" and "food plant" conidia, respectively,  
216 the difference in inactivation being 2.7 log between the two physiological states.

217 No significant impact was observed between the inactivation by sodium hypochlorite of the  
218 two physiological states of *P. commune* UBOCC-A-116003. For a contact time of 12.5 min at  
219 0.1% active chlorine, inactivation was 3 and 3.1 log for the "laboratory" and "food plant"  
220 spores, respectively. For a contact time of 7.5 min at 0.2% active chlorine, inactivation was  
221 3.1 and 3.5 log for "laboratory" and "food plant" physiological states, respectively. For *C.*  
222 *cladosporioides*, no significant difference was observed between the two physiological states  
223 at 0.1% active chlorine and 3% hydrogen peroxide. Indeed, at 0.1% active chlorine for 2.5  
224 min, the inactivation was 2.7 and 2.8 log for the "food plant" and "laboratory" spores,  
225 respectively, while, at 3% hydrogen peroxide for 60 min, inactivation was 1.2 and 1.4 log for  
226 the same physiological states, respectively.

227 As for the impact of physiological state after exposure to 0.2% active chlorine, it was  
228 impossible to determine as all conidia were inactivated from 2.5 min (inactivation > than 4.1  
229 log).

230 For *A. flavus*, the "food plant" conidia were more resistant than the "laboratory" spores for the  
231 two tested disinfectants. For a contact time of 12.5 min at 0.1% active chlorine, inactivation  
232 was 0.7 log and greater than 3.7 log for the "food plant" and "laboratory" conidia,  
233 respectively, difference in inactivation between the two physiological states being greater than  
234 3 log. For 0.2% active chlorine for 7.5 min, inactivation was 0.9 log and greater than 4.3 log  
235 for the "food plant" and "laboratory" conidia, respectively, and the difference in inactivation  
236 between the two physiological states was greater than 3.4 log. For a contact time of 15 min  
237 with 3% hydrogen peroxide, inactivation was 1.4 log and 3.6 log for the "food plant" and  
238 "laboratory" conidia, respectively. The difference in inactivation between the two  
239 physiological states was 2.2 log. Finally, for 3% hydrogen peroxide, the two *P. commune*

240 "food plant" conidia were more resistant than the "laboratory" ones. At a contact time of 10  
241 min, inactivation of *P. commune* UBOCC-A-112059 was nil and greater than 4.1 log for the  
242 "food plant" and "laboratory" conidia, respectively. The difference in inactivation between the  
243 two physiological states was greater than 4.1 log. For the same contact time, inactivation of *P.*  
244 *commune* UBOCC-A-116003 was 0.7 log and greater than 4.2 log for the "food plant" and  
245 "laboratory" conidia, respectively (difference in inactivation between the two physiological  
246 states being greater than 3.5 log).

247

#### 248 *3.4. Efficacy of fungicide molecules on the species*

249 With the exception of *C. cladosporioides* and *M. circinelloides*, a contact time of 5 min to  
250 0.1% active chlorine or 2.5 min to 0.2% active chlorine had almost no effect on the tested  
251 species. However, inactivation obtained for a contact time of 7.5 min to 0.2% active chlorine  
252 were of the same order of magnitude than treatments to 0.1% active chlorine for 12.5 min,  
253 except for "food plant" *P. commune* UBOCC-A-112059 conidia. However, these conditions  
254 were not sufficient to completely inactivate the two *P. commune* strains and the *A. flavus*  
255 "food plant" conidia. These conidia were the most resistant ones to sodium hypochlorite.

256 A contact time of 15 min to 3% hydrogen peroxide did not inactivate the spores of the tested  
257 species, except the "laboratory" *P. commune* conidia. In contrast to sodium hypochlorite, the  
258 *A. flavus* "food plant" conidia were not the most resistant ones to hydrogen peroxide.  
259 Surprisingly, *C. cladosporioides* and *M. circinelloides* exhibited a stronger resistance than the  
260 other species to a 15 min treatment to 3% hydrogen peroxide. Overall, at the respective tested  
261 concentrations, a longer time was required to inactivate the mold species with hydrogen  
262 peroxide than with sodium hypochlorite.

263

#### 264 4. Discussion

265 In order to assess a fungicidal efficacy, many studies examined the effect of disinfectant  
266 molecules for many contact times, but very few studies described inactivation kinetics. For  
267 chlorine inactivation, a classical linear decrease of the log survivors *versus* time with (for  
268 *Aspergillus fumigatus*) or without (for *Aspergillus versicolor* and *Penicillium purpurogenum*)  
269 an initial lag phase was observed (Ma and Bibby, 2017). In our study, a clear presence of an  
270 initial lag phase was shown for the inactivation of “food plant” *P. commune* conidia with  
271 0.1% active chlorine. Due to very fast inactivation kinetics leading to very few experimental  
272 data, our results did not show a clear linear decrease in the log survivors. However,  
273 inactivation curves exhibiting a tailing off (Pereira et al., 2013; Salomão et al., 2009) were not  
274 observed in our study. Inactivation kinetics in drinking water were evaluated by means of Ct  
275 (mg.min.l<sup>-1</sup>), i.e., concentration x time (Ma and Bibby, 2017, Pereira et al., 2013), these  
276 approaches assumed an inactivation rate proportional to the disinfectant concentration. For a  
277 given Ct value, inactivation should be the same regardless of the time and the concentration.  
278 In our study, inactivation for 0.1% active chlorine x 5 min and 0.2% active chlorine x 2.5 min  
279 were not significantly different regardless of the considered species or physiological state.  
280 Although this approach was not validated for other concentration x time conditions or other  
281 disinfectants.

282 Although the concept of dry-harvested spores was introduced as early as 1981 by Nickerson  
283 et al., almost all publications concern spores produced under optimal conditions and  
284 suspended into aqueous solutions (designated as “laboratory” spores in this study). In this  
285 context, Andrews (1986) found that 0.37% hypochlorite efficiently killed *A. flavus* after a 2  
286 min contact time. In another study, seven *A. flavus* isolates conidia were treated with a diluted  
287 commercial disinfectant for 72h. Five isolates did not show any survivors, i.e., log reduction  
288 greater than or equal to 2.7, for sodium hypochlorite concentrations in the range 0.4-1.6%.

289 However, the two other isolates exhibited survivors at 1.6% (Mattei et al., 2013). Eventually,  
290 4.3 log reduction for *A. flavus* conidia exposed to 3% hypochlorite (2.88% active chlorine) for  
291 10 min was reported by Bundgaard-Nielsen and Nielsen (1995). In our study, no survivors  
292 were detected after 10 min treatment to 0.2% active chlorine, thus suggesting that the *A.*  
293 *flavus* isolate tested in our study was more sensitive than that of the other study. This is a  
294 potential indication of behaviour heterogeneity within a given species.

295 The effect of sodium hypochlorite (0.1, 0.5 and 1%), was tested against *C.*  
296 *cladosporioides* for 15 min (Bernardi et al., 2018) exhibiting log reductions greater than 4. In  
297 our study no survivors (log reduction greater than 3.9) were detected for 5 min treatment at  
298 0.1%. As Bernardi et al. (2018) did not carry out treatment at contact times different than 15  
299 min, it is therefore difficult to compare. Noteworthy, these authors also tested *P. commune*  
300 conidia that exhibited 2-2.9 log reduction and more than 4 log reduction at 0.1% and 0.5/1%  
301 active chlorine, respectively. Our results showed 1.5 and 3 log reductions depending on the  
302 considered *P. commune* strain for 12.5 min treatments at 0.1% active chlorine. Therefore, the  
303 obtained results in this study are in accordance with those of Bernardi et al. (2018) despite  
304 slightly different contact times and methods (i.e., carrier method in Bernardi et al. (2018) and  
305 suspension method in this study). However, as previously mentioned for *A. flavus*,  
306 intraspecific diversity may also be an important factor to explain contrasted inactivation  
307 results, as 10 min treatments at 3% hypochlorite led to log inactivation in the range 1.7->5.2  
308 for other *P. commune* isolates (Bundgaard-Nielsen and Nielsen, 1995). In agreement with  
309 disinfectant susceptibility heterogeneity within a considered species, these authors also  
310 reported great differences of behaviour of five *Penicillium roqueforti* isolates when exposed  
311 to 0.3% hypochlorite for 10 min (log reductions ranged from 0.5 for the most resistant strain  
312 to 4.5 for the most sensitive one, while for the other strains, reductions were in the 1-2 log  
313 range).

314 For 15 min treatments with hydrogen peroxide (at 20°C), 3 log reduction in viable counts  
315 were obtained for 12.5%, *Absidia corymbifera*, 3.9%, *Candida albicans*, 5.51%,  
316 *Scopulariopsis brevicaulis*, 1.71%, *Aspergillus versicolor* and *Penicillium cyclopium*, and  
317 0.39%, *Geotrichum candidum* (Martin and Maris, 2012). In this study, 3 log and 3.7 log  
318 reduction of *A. flavus* conidia were obtained for 10 min and 15 min treatments with 3%  
319 hydrogen peroxide, respectively. For this species, a hydrogen peroxide concentration close to  
320 3% would have been required to obtain 3 log reduction in 15 min. According to our results,  
321 concentrations lower than (for both *P. commune* strains) and greater than (for *C.*  
322 *cladosporioides* and *M. circinelloides*) than 3% would have been necessary to obtain the same  
323 reduction.

324 In contrast to peroxide hydrogen, *P. commune* was more resistant than *C. cladosporioides* to  
325 active chlorine. This result is in accordance with the results reported by Bernardi et al. (2018)  
326 indicating, for 15 min treatments at 0.1% sodium hypochlorite, 2-2.9 log and more than 4 log  
327 reduction in *P. commune* and *C. cladosporioides* viable conidia, respectively. In accordance  
328 with Bundgaard-Nielsen and Nielsen (1995), *P. commune* was more resistant than *A. flavus* to  
329 active chlorine. Overall, both *P. commune* strains were more resistant to active chlorine than  
330 *A. flavus*, followed by *C. cladosporioides* and *M. circinelloides*. Surprisingly, the rank of  
331 resistance was the reverse than the one observed for hydrogen peroxide, *C. cladosporioides* >  
332 *M. circinelloides* > *A. flavus* > *P. commune*.

333 Noteworthy, the resistance ranking differed slightly for “food plant” spores. *A. flavus* was the  
334 more resistant and the more sensitive species to active chlorine and hydrogen peroxide,  
335 respectively. The rank of resistance depended also on the considered strain; indeed, whereas  
336 *P. commune* UBOCC-A-116003 was more resistant than the UBOCC-A-112059 to 70%  
337 ethanol (Visconti et al., 2020), UBOCC-A-112059 was more resistant than UBOCC-A-  
338 116003 to 3% hydrogen peroxide. This study also showed that differences in resistance

339 depended on the concentration of the disinfectant, at 0.1% active chlorine no difference was  
340 noticed between the two *P. commune* strains, while, at 0.2% active chlorine, UBOCC-A-  
341 116003 was more resistant than UBOCC-A-112059. At the tested concentrations in this study,  
342 active chlorine was more efficient than hydrogen peroxide. In this study, *P. commune*  
343 UBOCC-A-112059 “laboratory” conidia were more resistant than the other isolate to active  
344 chlorine, but less resistant than the other isolate to hydrogen peroxide. The impact of the  
345 physiological state was greater than 1.54 log inactivation for *P. commune* UBOCC-A-116003  
346 treated with 70% ethanol (Visconti et al., 2020), but the impact was even greater than 3.5 log  
347 for 10 min contact time to 3% peroxide hydrogen. “Food plant” *A. flavus* conidia were also  
348 more resistant than “laboratory” ones, but *M. circinelloides* “laboratory” spores were more  
349 resistant to all disinfectants than the “food plant” ones. In contrast to the other species, *M.*  
350 *circinelloides* can hardly develop at water activities below 0.90  $a_w$  (Hocking and Miscamble,  
351 1995; Tresner and Hayes, 1971) and at 0.95  $a_w$ , the growth rate was shown to about half the  
352 optimum growth rate (Morin-Sardin et al., 2016). In contrast, *A. flavus* is a xerophilic species  
353 that can grow at water activities less than 0.85  $a_w$ , (Pitt and Miscamble, 1995; Sautour et al.,  
354 2001, 2002). At 0.95  $a_w$  the growth rate was almost the same than that observed at the  
355 optimum water activity of 0.974 (Sautour et al., 2001). These observations suggested that the  
356 increased sensitivity to disinfectants of *M. circinelloides* “food plant” spores may be due to  
357 the fact that these spores were produced at a water activity (0.95  $a_w$ ) too close to the minimum  
358 one.

359

## 360 **5. Conclusions**

361 Surface disinfection in food industries should be based on the use of most effective sanitizer,  
362 for the best “contact time / concentration” combination and taking into account the most  
363 resistant mold, in agreement with a “worst case scenario” approach. In many cases, a lag



364 phase prior to inactivation was observed. Therefore, shortening the contact time could lead to  
365 the elimination of the disinfectant even before fungal spores are actually killed. What can be  
366 considered as the most resistant mold depends not only on the encountered species and strains  
367 within a given species (disinfectant susceptibility heterogeneity), but also, as demonstrated in  
368 this study, on the physiological state of fungal spores and even on the considered disinfectant  
369 molecule. Based on the obtained data, it is recommended to assess fungicidal activity of  
370 disinfectants on “food plant” spores, not only because there were more resistant than  
371 “laboratory” ones for the most resistant species, but because the “food plant” physiological  
372 state was closer to airborne conidia that are responsible for actual food contamination and  
373 spoilage.

374

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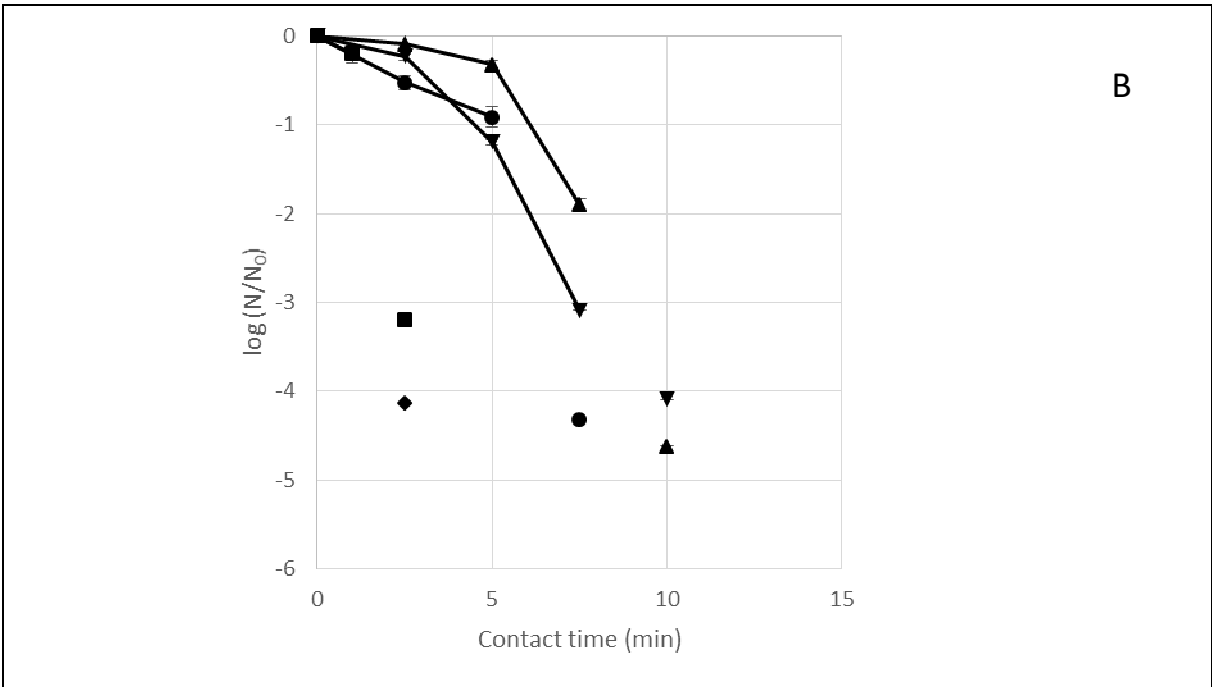
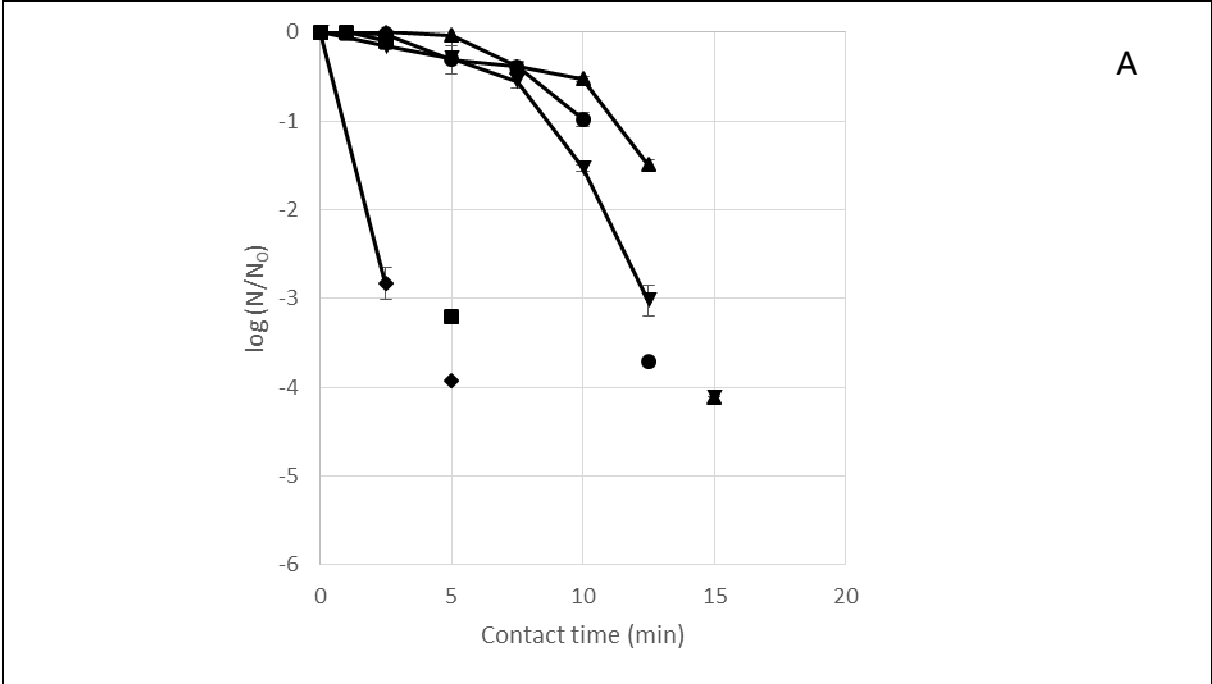
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## Figure legends

**Fig. 1.** Inactivation of “laboratory” spores (produced at 0.99  $a_w$  and harvested using an aqueous solution) of *Aspergillus flavus* (●), *Cladosporium cladosporioides* (◆), *Mucor circinelloides* (■), *Penicillium commune* (UBOCC-A-112059) (▲) and *Penicillium commune* (UBOCC-A-116003) (▼). Treatment with A) 0.1% active chlorine, B) 0.2% active chlorine and C) 3% hydrogen peroxide. Linked symbols = presence of survivors and isolated symbol = absence of survivors. Error bars represent standard deviations.

**Fig. 2.** Inactivation of “food plant” spores (produced at 0.95  $a_w$  and dry-harvested) of *Aspergillus flavus* (⊙), *Cladosporium cladosporioides* (◇), *Mucor circinelloides* (□), *Penicillium commune* (UBOCC-A-112059) (▲) and *Penicillium commune* (UBOCC-A-116003) (▼). Treatment with A) 0.1% active chlorine, B) 0.2% active chlorine and C) 3% hydrogen peroxide. Linked symbols = presence of survivors and isolated symbol = absence of survivors. Error bars represent standard deviations.





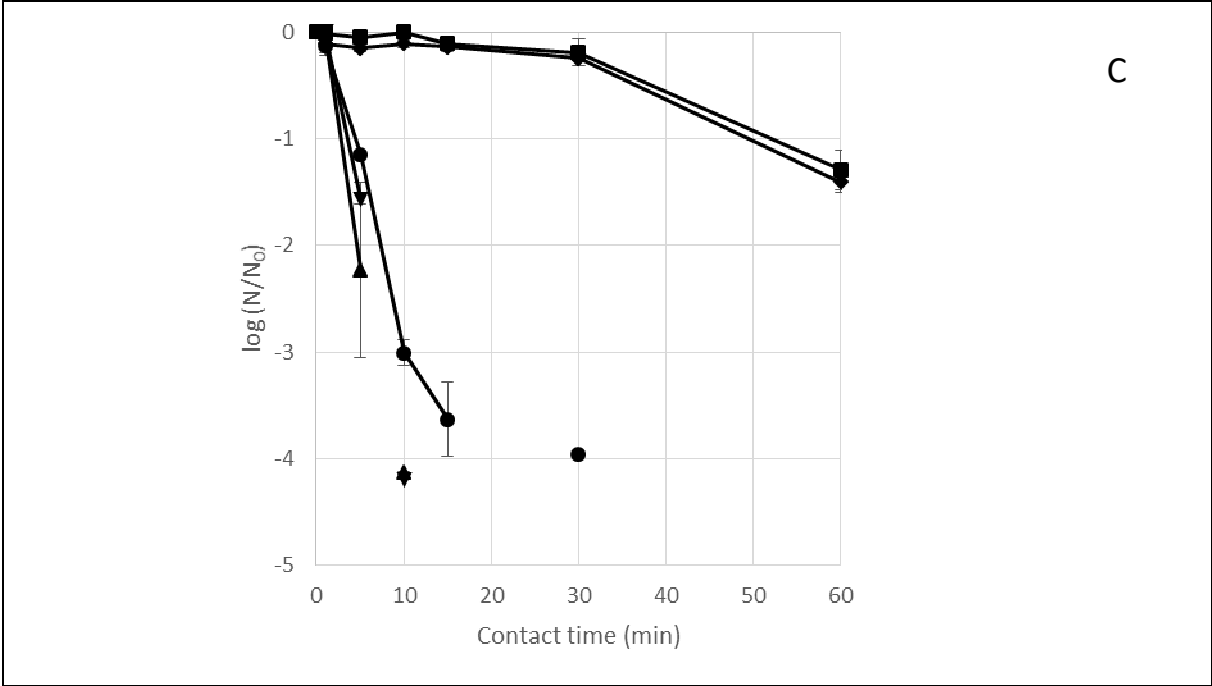
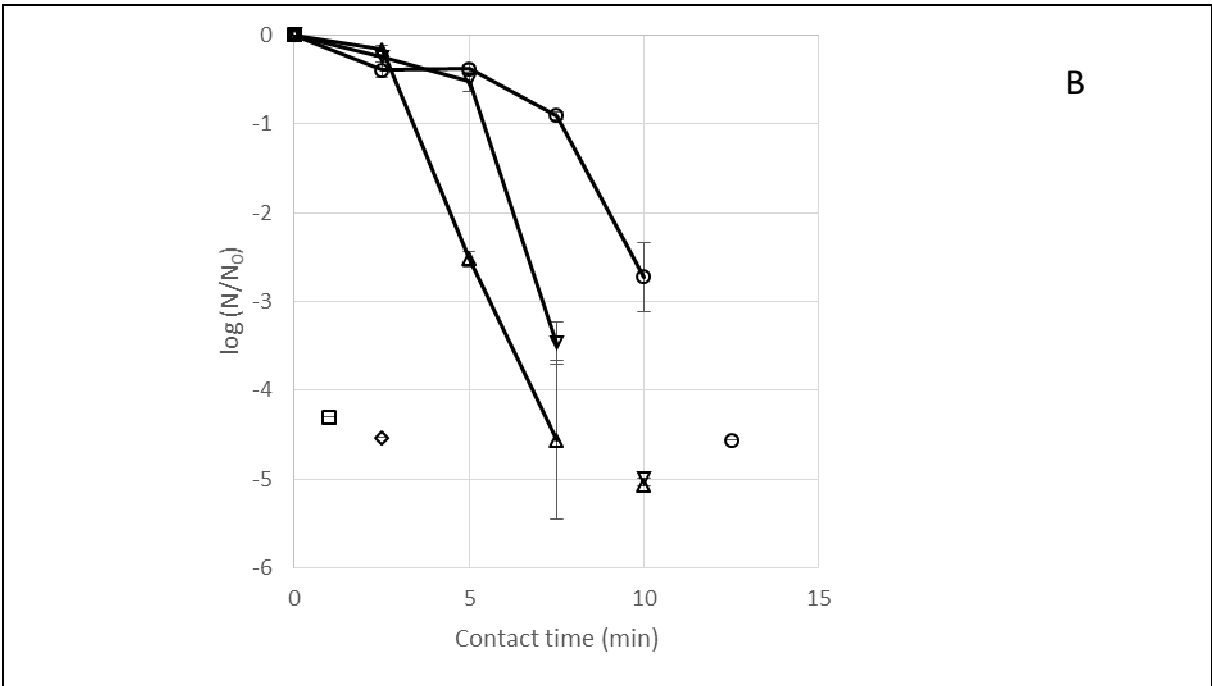
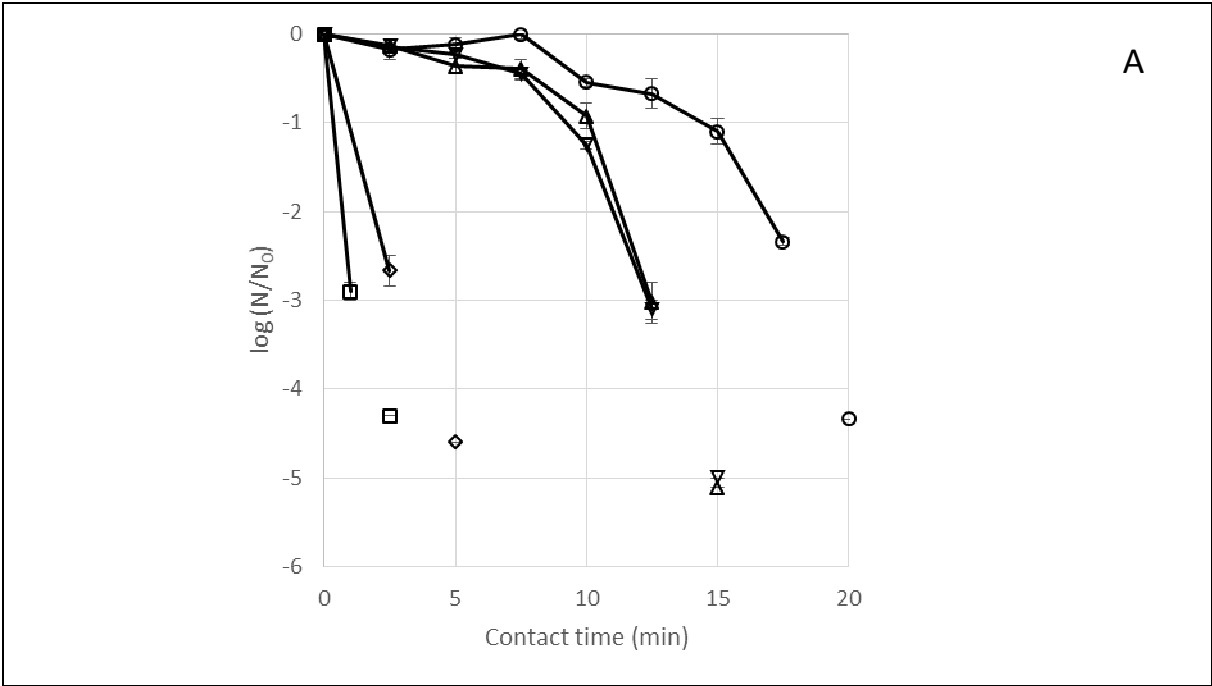


Figure 1



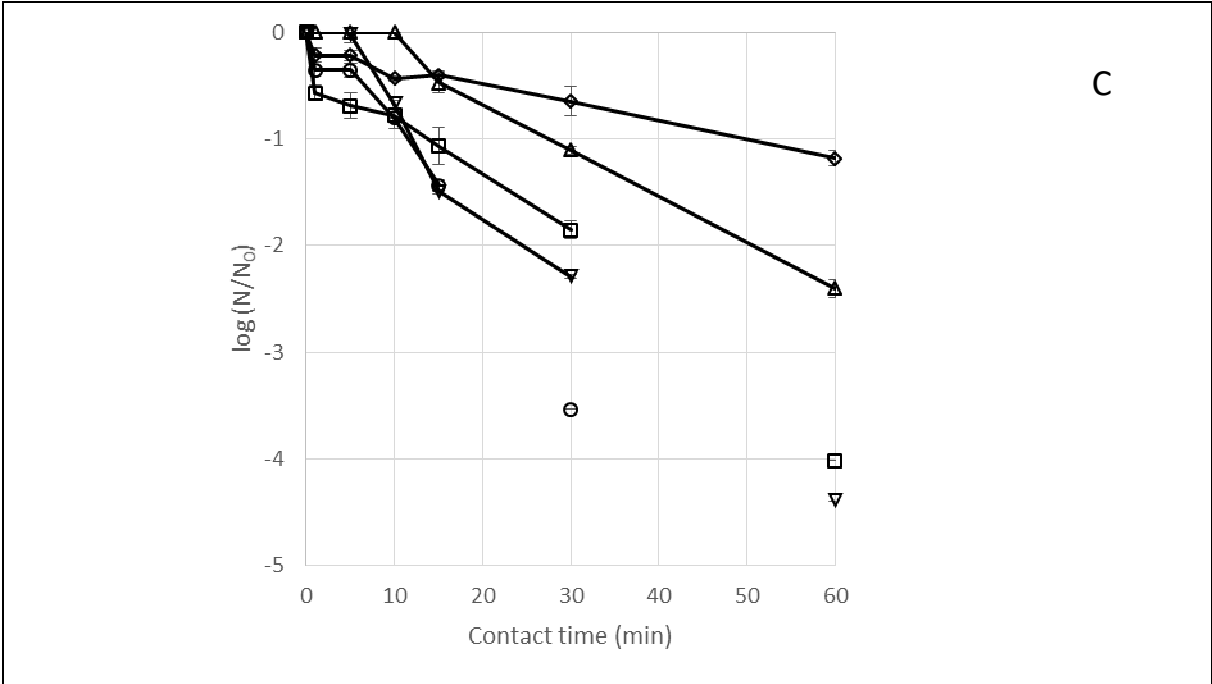


Figure 2

**Table 1. List of strains provided by the Université de Bretagne Occidentale Culture Collection (UBOCC).**

Species	Reference collection number	Origin
<i>Aspergillus flavus</i> var <i>columnaris</i>	UBOCC-A-108066	Butter
<i>Cladosporium cladosporioides</i>	UBOCC-A-111114	Fresh dairy product
<i>Mucor circinelloides</i>	UBOCC-A-112187	Yoghurt
<i>Penicillium commune</i>	UBOCC-A-112059	Blue-veined cheese
<i>Penicillium commune</i>	UBOCC-A-116003	Blue-veined cheese

**Table 2. Log<sub>10</sub> reductions obtained after exposure to various disinfectants using the liquid method.**

Mold (Strain)		Inactivation values (log <sub>10</sub> )									
		<i>Aspergillus flavus</i> (UBOCC-A-108066)		<i>Cladosporium cladosporioides</i> (UBOCC-A-111114)		<i>Mucor circinelloides</i> (UBOCC-A-112187)		<i>Penicillium commune</i> (UBOCC-A-112059)		<i>Penicillium commune</i> (UBOCC-A-116003)	
Physiological state		Labo- ratory	Food plant	Labo- ratory	Food plant	Labo- ratory	Food plant	Labo- ratory	Food plant	Labo- ratory	Food plant
Sodium hypochlorite 0.1% active chlorine	2.5 min	0.0 (± 0.1)	0.2 (± 0.1)	2.8 (± 0.2)	2.7 (± 0.2)	0.1* (± 0.1)	> 4.3*	0.0 (± 0.1)	0.1 (± 0.1)	0.2 (± 0.1)	0.1 (± 0.1)
	5 min	0.2 (± 0.1)	0.1 (± 0.1)	> 3.9	> 4.6	> 3.2	> 4.3	0.0 (± 0.2)	0.4 (± 0.1)	0.3 (± 0.1)	0.2 (± 0.1)
	12.5 min	> 3.7*	0.7* (± 0.2)						1.5* (± 0.1)	3.0* (± 0.2)	3.0 (± 0.2)
Sodium hypochlorite 0.2% active chlorine	1 min					0.2* (± 0.1)	> 4.3*				
	2.5 min	0.5 (± 0.1)	0.4 (± 0.1)	> 4.1	> 4.5	> 3.2	> 4.3	0.1 (± 0.1)	0.2 (± 0.1)	0.2 (± 0.1)	0.2 (± 0.1)
	7.5 min	> 4.3*	0.9* (± 0.1)					1.9* (± 0.1)	4.6* (± 0.9)	3.1 (± 0.1)	3.5 (± 0.2)
Hydrogen peroxide 3%	10 min	3.0* (± 0.1)	0.8* (± 0.1)	0.1 (± 0.1)	0.4 (± 0.1)	0.0* (± 0.1)	0.8* (± 0.1)	> 4.1*	0.0* (± 0.1)	> 4.2*	0.7* (± 0.1)
	15 min	3.6* (± 0.4)	1.4* (± 0.1)	0.1 (± 0.1)	0.4 (± 0.1)	0.1* (± 0.1)	1.1* (± 0.2)	> 4.1*	0.5* (± 0.1)	> 4.2*	1.5* (± 0.1)
	60 min	> 4.0	> 3.5	1.4 (± 0.1)	1.2 (± 0.1)	1.3* (± 0.2)	> 4.0*	> 4.1*	2.4* (± 0.1)	> 4.2	> 4.4

“Laboratory”: spores produced at 0.99  $a_w$  and harvested using an aqueous solution. “Food plant”: spores produced at 0.95  $a_w$  and dry-harvested. Shaded cells were used to compare the physiological states. \*: inactivation of “laboratory” spores significantly different from that of “food plant” ones at p=0.05.