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1 **1-octanol, a self-inhibitor of spore germination in *Penicillium***
2 ***camemberti***

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4 Guillaume Gillot¹, Nicolas Decourcelle², Gaëlle Dauer², Georges Barbier¹, Emmanuel Coton¹,
5 David Delmail³, Jérôme Mounier^{1*}.

6
7 ¹ *Université de Brest, EA 3882 Laboratoire Universitaire de Biodiversité et Ecologie*
8 *Microbienne, ESIAB, Technopôle Brest-Iroise, 29280 Plouzané, France.*

9 ² *Université de Brest, EA 3882 Laboratoire Universitaire de Biodiversité et Ecologie*
10 *Microbienne, IUT de Quimper, 2, rue de l'Université 29200, Quimper, France*

11 ³ *Université de Rennes 1, UEB, UMR CNRS 6226 ISCR PNSCM, 2 avenue du Professeur*
12 *Léon Bernard, 35043 Rennes, France.*

13
14 **Running title:** Quorum sensing in *Penicillium camemberti*

15
16 ***Corresponding author:** Jérôme Mounier

17 EA3882 - Laboratoire Universitaire de Biodiversité et Ecologie Microbienne,

18 Parvis Blaise Pascal, Technopôle Brest-Iroise

19 29280 Plouzané, France

20 Tel: +33 (0)2.90.91.51.10

21 Fax: +33 (0)2.90.91.51.01

22 E-mail: jerome.mounier@univ-brest.fr

23

24 **Abstract**

25 *Penicillium camemberti* is a technologically relevant fungus used to manufacture mould-
26 ripened cheeses. This fungal species produces many volatile organic compounds (VOCs)
27 including ammonia, methyl-ketones, alcohols and esters. Although it is now well known that
28 VOCs can act as signaling molecules, nothing is known about their involvement in *P.*
29 *camemberti* lifecycle. In this study, spore germination was shown to be self-regulated by
30 quorum sensing in *P. camemberti*. This phenomenon, also called the "crowding effect", is
31 population-dependent (*i.e.* observed at high population densities). After determining the
32 volatile nature of the compounds involved in this process, 1-octanol was identified as the
33 main compound produced at high-spore density using GC-MS. Its inhibitory effect was
34 confirmed *in vitro* and 3 mM 1-octanol totally inhibited spore germination while 100 μ M only
35 transiently inhibited spore germination. This is the first time that self-inhibition of spore
36 germination is demonstrated in *P. camemberti*. The obtained results provide interesting
37 perspectives for better control of mould-ripened cheese processes.

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39
40 **Keywords:** Cheese, quorum sensing, *Penicillium camemberti*, germination, 1-octanol.

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42
43 **Chemical compounds studied in this article:** 1-octanol (PubChem CID: 957), ethanol
44 (PubChem CID: 702), ammonia (PubChem CID: 222), 2-heptanone (PubChem CID: 8051),
45 2-nonanone (PubChem CID: 13187), 1-octen-3-ol (PubChem CID: 18827), trans-2-octen-1-ol
46 (PubChem CID: 5318599), 2-methyl-1-butanol (PubChem CID: 8723), 3-methyl-1-butanol
47 (PubChem CID: 31260) and 3-octanone (PubChem CID: 246728).

48

49 1. Introduction

50 Fungal spores can be dispersed in different ways, namely through air, dust or water, and are
51 important for fungal dissemination and/or survival. Conidia are dispersed asexual spores and
52 can be released in massive numbers by fungi, in particular by *Penicillium* genus members
53 (Dijksterhuis and Samson, 2002) including many food-related species. While conidia are
54 easily disseminated, spore germination only occurs when favorable environmental conditions
55 (*i.e.* temperature, pH, humidity, light and nutrients) are met. Spore germination is also
56 influenced by spore density and is hindered at high spore densities (Macko et al., 1972). First
57 evidence for this phenomenon, also called “quorum sensing”, was shown for genetic
58 competence induction (Felkner and Wyss, 1964; Tomasz and Hotchkiss, 1964) and in light
59 production regulation (Nealson and Hastings, 1979) in Gram-positive bacteria and marine
60 vibrios, respectively. The ability of microbial cells to chemically sense the density of the
61 surrounding population (Fuqua et al., 1994) by extracellular factors has also been found in
62 diverse microorganisms including fungi (Hogan, 2006). This phenomenon actually allows for
63 cell density-dependent growth regulation, hence the term "crowding effect", and appears to be
64 prevalent in diverse fungal species (Hogan, 2006). Previous studies suggested that
65 intercellular signaling via self-inhibitor compound production prevents premature germination
66 and guarantees that spores only germinate at the suitable time, *i.e.* in favorable environmental
67 conditions.

68 Self-inhibitors, which can correspond or not to volatile compounds, have been characterized
69 in numerous fungal genera including *Aspergillus*, *Colletotrichum*, *Dictyostelium*, *Fusarium*,
70 *Glomerella*, *Penicillium*, *Puccinia*, *Syncephalastrum* and *Uromyces* (Bacon et al., 1973;
71 Barrios-González et al., 1989; Chitarra et al., 2004; Garrett and Robinson, 1969; Hobot and
72 Gull, 1980; Lax et al., 1985; Leite and Nicholson, 1992; Lingappa et al., 1973; Macko et al.,
73 1972). Regarding the *Penicillium* genus, Chitarra et al. (2004, 2005) demonstrated that 1-

74 octen-3-ol influenced different developmental processes during the *Penicillium paneum*
75 lifecycle, including spore germination inhibition.

76 *Penicillium camemberti*, first described by Thom in 1906 (Raper and Thom, 1949), is mainly
77 used in cheese manufacture and more particularly for mould-ripened soft cheeses such as
78 Camembert, Brie and Coulommiers. *P. camemberti* growth results in the formation of a
79 characteristic white rind (Abbas and Dobson, 2011). During spore germination and mycelial
80 growth, lipase and protease activities are involved in lipid and protein degradation in cheese
81 resulting in fatty and amino acid release (Beresford and Williams, 2004). These latter are then
82 transformed into important taste and aroma compounds such as ammonia, methyl-ketones,
83 primary and secondary alcohols, esters, aldehydes, lactones and sulfur compounds (Abbas and
84 Dobson, 2011) and contribute to overall organoleptic qualities.

85 Due to the important role of *P. camemberti* in cheese manufacture, there is a clear interest to
86 understand regulatory mechanisms potentially involved in conidia germination to better
87 control soft mould-ripened cheese production. In this context, the aims of this study were to
88 first investigate the effect of spore density on spore germination in *P. camemberti*, then to
89 identify the molecules involved and finally estimate the impact of the identified compound
90 and other volatile compounds on spore germination.

91

92 **2. Materials and methods**

93 **2.1 Strains, culture conditions and spore suspension preparations**

94 Two commercial *P. camemberti* strains (coded *P. camemberti* A and B for confidentiality
95 reasons) isolated from mould-ripened cheeses and *P. camemberti* UBOCC-A-101392 and
96 UBOCC-A-108097, and CBS 299.48^T respectively obtained from the Université de Bretagne
97 Occidentale Culture Collection (UBOCC, Plouzané, France) and the Central Bureau Voor
98 Schimmelcultures (Baarn, Netherlands) were used in this study. Pre-cultures were prepared

99 on slant Potato Dextrose Agar (Difco PDA, Becton, Dickinson and Company, Franklin Lakes,
100 NJ, USA) by inoculating a spore suspension, conserved at -80°C in 10 % glycerol (v/v), and
101 incubating tubes for 7 days at 25°C . Strains were then sub-cultured on slant PDA and
102 incubated at 25°C for 14 days. After incubation, spores were harvested using 8 mL of sterile
103 distilled water supplemented with 0.1 % Tween 80. Slant agar cultures were scraped using a 1
104 mL sterile pipette to collect spores. Then, Roux flasks containing 100 mL Malt Extract Agar
105 (MEA, Merck, Darmstadt, Germany) were sowed with 6 mL spore suspension. After
106 incubation for 14 days at 25°C , 25 mL of sterile distilled water supplemented with 0.1 %
107 Tween 80 were added to each Roux flask and the mycelium surface was scraped using a cell
108 scraper (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The resulting spore
109 suspension was transferred into a tube and centrifuged for 5 min at 4000 g at 4°C (CR3i
110 multifunction, Thermo Fisher Scientific Inc., Waltham, MA, USA). After carefully discarding
111 the supernatant, spores were resuspended in 5 mL sterile distilled water supplemented with
112 0.1 % of Tween 80. Finally, all suspensions of a same strain were pooled together, counted
113 using a Malassez cell and standardized to $2 \cdot 10^8$ and $2 \cdot 10^6$ spores.mL⁻¹.

114

115 **2.2 Effect of spore concentration on germination kinetics in culture broth**

116 Spore germination kinetics were performed as described by Chitarra et al. (2004, 2005) with
117 some modifications in Malt Extract Broth (MEB, Merck, Darmstadt, Germany) using fresh
118 spore suspensions. To determine the effect of spore density on germination kinetics, 0.75 mL
119 spore suspensions (either $2 \cdot 10^8$ or $2 \cdot 10^6$ spores.mL⁻¹) were mixed with 0.75 mL MEB 2X to
120 obtain a final spore concentration of 10^8 or 10^6 spores.mL⁻¹. Suspensions were then incubated
121 horizontally in a rotary shaker set to 25°C and 120 rpm. Germination was followed at 0 h, 8 h,
122 then at 2 h intervals for the following 12 h. A minimum of 100 conidia were counted to
123 determine germination kinetics (i.e. percentage of germinated spore as a function of time). A

124 spore was considered as germinated when the germinal tube length was superior or equal to
125 the spore diameter itself, as previously described (Dantigny et al., 2006). In addition, in order
126 to examine whether spore germination capacity was related to spore density, high density
127 spore suspensions obtained after 20 h incubation were diluted to 10^6 spores.mL⁻¹ in fresh
128 MEB 1X and incubated for another 18 h. Moreover, to assess whether inhibitory molecules
129 were produced during incubation at high spore density, filter sterilized cultures obtained after
130 20 h incubation were inoculated at 10^6 spores.mL⁻¹ and also incubated for another 18 h. For
131 each spore concentration level, three technical replicates and two biological replicates were
132 performed.

133

134 **2.3 Effect of spore density on spore germination and radial growth in agar medium**

135 In these experiments, a Petri dish containing Malt Extract Agar (MEA, Merck, Darmstadt,
136 Germany) layers at the bottom and the top (in the lid) was used as described previously
137 (Chitarra et al., 2004). The top MEA layer was surface inoculated with 50 μ L of a 10^6 or 10^8
138 spores.mL⁻¹ suspension while 5 μ L of a 10^7 spores.mL⁻¹ suspension were deposited into the
139 center of the plate on the bottom layer. Control cultures were also performed without
140 inoculating the top layer. Colony diameters were measured in two perpendicular directions
141 every 2 days for 14 days (plates were incubated at 25°C). Five replicates per condition were
142 performed. Mean values \pm 95 % confidence intervals were calculated for each condition.

143

144 **2.4 Extraction of volatile compounds**

145 Volatile compounds produced by *P. camemberti* in liquid cultures after 20 h incubation were
146 extracted as previously described (Husson et al., 2002). Briefly, 0.5 g NaCl and 1 mL diethyl
147 ether were added to 1 mL culture suspension followed by centrifugation at 15,000 g for 2 min

148 at room temperature. The organic phase (superior phase) was recuperated and Na₂SO₄ was
149 added to remove any traces of water.

150

151 **2.5 Detection and identification of volatile compound(s) by GC-MS**

152 Detection and identification of volatile compounds were performed by gas chromatography
153 (Agilent Technologies 7820A GC Systems, Agilent Technologies, Santa Clara, CA, USA)
154 coupled with mass spectrometry (Agilent Technologies 5975 Series MSD, Agilent
155 Technologies, Santa Clara, CA, USA) using a HP-5ms (30 m x 250 µm x 0.25 µm) column.
156 The program used for the oven was as follows: 50°C for 5 min, 4°C.min⁻¹ between 50°C and
157 200°C then 10°C.min⁻¹ from 200°C to 270°C, followed by a final step at 270°C for 10 min.
158 The injector and detector temperatures were set to 250°C and 300°C, respectively. The
159 conditions of the mass spectrometer were mode electronic impact (EI); temperature source
160 250°C; scanning speed 1 scan.s⁻¹; mass acquisition 50-300 uma. The vector gas was H₂ at a
161 1.5 mL.min⁻¹ flow rate. For identification, mass spectra were compared to the NIST mass
162 spectral library and were confirmed using the retention index and mass spectrum of pure
163 compounds.

164

165 **2.6 Effect of pure volatile compounds on spore germination in MEB medium**

166 The impact of the major volatile compound identified by GC-MS on the spore germination
167 process was evaluated in MEB for two strains (*P. camemberti* A and B) at concentrations
168 ranging between 0 and 4 mM. The effect of other volatile compounds potentially produced by
169 *P. camemberti*, i.e. ethanol, ammonia, 2-heptanone, 2-nonanone, 1-octen-3-ol, trans-2-octen-
170 1-ol, 2-methyl-1-butanol, 3-methyl-1-butanol and 3-octanone, was also investigated. To
171 perform these assays, after solubilization of the tested compound in propylene glycol and
172 serial dilutions, the percentage of germinated conidia from a 10⁶ spore.mL⁻¹ suspension was

173 determined in MEB as described above, after 10 and 16h incubation at 25°C with agitation
174 (120 rpm). Control cultures without volatile compound but containing the same amount of
175 propylene glycol were also performed.

176

177 **2.7 Effect of initial spore concentration on the growth of *P. camemberti* determined by** 178 **ergosterol analysis in a cheese matrix model**

179 The cheese matrix model was prepared as described previously (Le Dréan et al., 2010).
180 Briefly, to prepare 100 g, 23 g milk protein concentrate, 20 g anhydrous milk fat, 50 mL
181 sterile distilled water, 1.5 g NaCl and 1.26 mL lactic acid were added. Prepared model
182 cheeses were inoculated with either *P. camemberti* A or B spore suspensions to yield a final
183 spore concentration ranging from 10^2 to 10^6 spores.g⁻¹. Then, 50 g cheese portions were
184 transferred to sterile crystallizing dishes (5.6-cm diameter) and incubated for 9 days at 12°C.
185 After incubation, a 32-mm diameter and ~5 mm depth layer was removed from the cheese
186 surface with a sterile scalpel. Sufficient sterile 2% (w/v) trisodium citrate was added to yield a
187 1:10 dilution and the resulting suspension was homogenized with an Ultra-Turrax (IKA,
188 Staufen, Germany) at 24,000 rpm.min⁻¹ for 1 min. Five mL of the trisodium citrate / cheese
189 mixture were then centrifuged in a 12-mL screw cap tube at 10,000 g for 10 min at 4°C. The
190 supernatant was discarded and the mycelium pellets resuspended in 0.5 mL of methanol.
191 After vortexing, 2.5 mL of 24 % methanolic KOH (24 %) were added and incubated for 2h at
192 85°C. After cooling in melted ice for 10 min, 2 mL of petroleum ether were added and
193 vortexed 3 times for 20 sec with a cooling step on ice for 2 min after each homogenization
194 treatment. This step was repeated and the extract was centrifuged for 10 min at 3000 rpm at
195 4°C. The organic phase was collected and evaporated under nitrogen flow for ~15 min and
196 stored at -20°C before use. Prior to HPLC analysis, the dry extract was dissolved in 0.5 mL
197 methanol and filtered through a 0.45-µm acetate filter. Ergosterol was quantified, using

198 external ergosterol standards (Sigma, St Louis, MO, USA), with a high-performance liquid
199 chromatograph Agilent 1100 series (Agilent Technologies, Santa Clara, CA, USA) equipped
200 with an Interchrom Lichrospher C18 column and an UV detector set at 282 nm. The mobile
201 phase was methanol with a flow rate set to 1.2 mL.min⁻¹ and injection volume was 50 µL.
202 Each sample and standard were analyzed in triplicate. Mean values ± 95 % confidence
203 intervals were calculated for each strain and condition.

204

205 **3. Results**

206 **3.1 Effect of spore concentration on germination kinetics in broth medium**

207 Germination kinetics according to initial spore population for the 2 studied *P. camemberti*
208 strains in MEB are presented in Fig. 1. Independently of the studied strains, cultures
209 containing 10⁶ spores.mL⁻¹ presented higher germination percentages compared to 10⁸
210 spores.mL⁻¹ cultures. Indeed, after 20h incubation, >90 % and <3% spores had germinated at
211 10⁶ versus 10⁸ spores.mL⁻¹. Similar results were also obtained on *P. camemberti* strains
212 UBOCC-A-101392, UBOCC-A-108097 and CBS 299.48^T (data not shown). To determine
213 whether germination inhibition was the consequence of higher spore density, 10⁸ spores.mL⁻¹
214 cultures obtained after 20 h incubation were diluted to 10⁶ spores.mL⁻¹ and further incubated
215 for 18 h. Germination percentages for both strains were ~ 90 % indicating that a "crowding-
216 effect" was responsible for this inhibition.

217 In order to check for self-inhibitory molecules in the high density spore suspension, 20-h 10⁸
218 spores.mL⁻¹ culture filtrates were inoculated with a new spore suspension (10⁶ spores.mL⁻¹)
219 and germination kinetics were compared to a control (Fig. 2). While 98 % of spores had
220 germinated in the control, germination rates in the culture filtrates were only 13 % and 8 %
221 for *P. camemberti* A and B, respectively (Fig. 2), thus, highlighting that one or several self-
222 inhibitors were present in the culture medium previously containing 10⁸ spores.mL⁻¹.

223

224 **3.2 Effect of spore density on spore germination and radial growth in agar medium**

225 To examine if spore density could impact spore germination and radial growth in solid
226 medium, a Petri dish containing MEA layers at both the bottom and top (in the lid) was used
227 as previously described by Chitarra et al. (2004, 2005). As shown in Fig. 3, growth in the
228 bottom of the Petri dish was significantly delayed as compared to the control (Fig. 3A) when
229 the lid was inoculated with 50 μL of a spore suspension containing 10^6 (Fig. 3B) or 10^8 (Fig.
230 3C) spores.mL⁻¹, the latter having the strongest effect. Indeed, mean radial growth rates were
231 4.14, 0.57 and 0.47 mm.d⁻¹ for the control and the lid agar inoculated with 10^6 and 10^8
232 spores.mL⁻¹, respectively. Overall, these results strongly suggested that volatile compounds,
233 able to move from the MEA layer of production to the MEA layer of action, could be
234 responsible for the observed effects.

235

236 **3.3 Detection and identification of volatiles compound(s) by GC-MS**

237 The volatile compounds present in MEB cultures containing 10^8 spores.mL⁻¹ of *P.*
238 *camemberti* strain A or B were analyzed by GC/MS and compared to those of the non-
239 inoculated MEB medium (Fig. 4). Major differences in volatile compound profiles between
240 the inoculated media (Fig. 4A) and control (Fig. 4B) were observed. Noteworthy, the most
241 abundant volatile compound produced at high spore density exhibited a retention time of
242 10.997 min. The nature of this compound was assigned to 1-octanol based on its mass
243 spectrum (Fig. 4C) and confirmed after comparison of its mass spectrum with that of pure 1-
244 octanol (Fig. 4D). This compound was also verified to have a different retention time and
245 mass spectrum from those of 1-octen-3-one, 3-octanone, 2-octanone, octanal, trans-2-octen-1-
246 ol and 1-octen-3-ol (retention times were 7.237, 8.03, 8.201, 8.488, 11.935 and 12.796 min,
247 respectively) in the tested conditions (data not shown).

248

249 **3.4 Effect of pure volatile compounds on germination efficiency in MEB**

250 The effects of 1-octanol, as well as other volatile compounds potentially produced by *P.*
251 *camemberti*, on germination efficiency were studied *in vitro*. The germination inhibitory
252 effect of 1-octanol was confirmed in MEB. Indeed, total inhibition of spore germination was
253 achieved in the presence of 3 mM 1-octanol while a concentration as low as 100 μ M
254 transiently delayed spore germination after 10h but not after 16h. Indeed, at the latter
255 concentration, the percentage of germinated spores was reduced by 10 % as compared to the
256 control. It is worth mentioning that 1-octen-3-ol, a compound previously identified to act as a
257 self-inhibitor in *P. paneum* (Chitarra et al., 2005, 2004), and its isomer trans-2-octen-1-ol had
258 a similar minimum inhibitory concentration (MIC) than 1-octanol. Other compounds, namely,
259 ethanol, ammonia, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-heptanone and 3-octanone did
260 not inhibit spore germination in the tested conditions (MIC>4 mM).

261

262 **3.5 Effect of spore concentration on *P. camemberti* growth as determined by** 263 **ergosterol analysis in a cheese matrix model**

264 Fungal biomass obtained after 9 days incubation at 12 °C as a function of the initial spore
265 concentration (10^2 to 10^6 spores.g⁻¹ of cheese) is shown in Fig. 5. Independently of the tested
266 strain, there was no significant difference in fungal biomass whatever the initial spore
267 concentration used.

268

269 **4. Discussion**

270 Self-regulation of spore germination as a function of spore density has been previously
271 characterized in a wide range of fungal species including *Penicillium* species (Chitarra et al.,
272 2004, 2005). In the present study, we demonstrated for the first time that this phenomenon,

273 which was reversible, also occurred in *P. camemberti*, an industrially relevant fungus used in
274 the dairy industry. We also showed that 1-octanol, a volatile compound, was produced at high
275 spore density and that this compound hindered spore germination *in vitro*. In nature,
276 *Penicillium* spp. produce high spore numbers which are directly exposed to air, thus
277 explaining why volatile self-inhibitors may be more widespread than non-volatile ones. Thus,
278 as suggested by Chitarra et al. (2004) for 1-octen-3-ol, we can hypothesize that 1-octanol is
279 produced by *P. camemberti* conidia and released into the air in order to inhibit germination
280 until appropriate environmental conditions are met. Likewise, as previously described for 1-
281 octen-3-ol in *P. paneum* and *Aspergillus nidulans* (Chitarra et al., 2005; Herrero-Garcia et al.,
282 2011), conidia treated with 3 and 4 mM 1-octanol did not enter into isotropic growth,
283 meaning that 1-octanol prevented the initiation of the spore swelling process which precedes
284 polarized growth and the formation of a germ tube. Thus, this volatile compound may have an
285 effect on fungal membrane permeabilisation which controls water entry into conidia (Chitarra
286 et al., 2005).

287 More generally, eight-carbon (8-C) volatiles are ubiquitous among fungi and characteristic of
288 fungal aromas (Combet et al., 2006). They result from the oxidation and cleavage of fatty
289 acids, in particular linoleic acid. Such products constitute for 44.3- 97.6% of the total amount
290 of volatiles produced, depending on the extraction method used (Maga, 1981; Tressl et al.,
291 1982; Venkateshwarlu et al., 1999). The well-known 1-octen-3-ol presenting a mushroom-
292 like aroma is the most abundant VOC while 1-octanol is characterized by a detergent, soap
293 and orange-like aroma (Combet et al., 2006). In the present study, 1-octanol was the only 8-C
294 volatile compound identified in cultures with high conidial density while 1-octen-3-ol was not
295 detected. Likewise, *P. commune*, which is considered as an ancestral form of the domesticated
296 *P. camemberti* (Pitt et al., 1986), was not found to produce 1-octen-3-ol at high spore density
297 (Chitarra et al., 2005). Nevertheless, 1-octen-3-ol was found, here, to block the germination

298 process *in vitro*. Given that the role of this compound as a self-inhibitor of germination has
299 also been demonstrated in several fungal genera and species (Chitarra et al., 2004; Herrero-
300 Garcia et al., 2011), it could be considered as a cross-talk molecule. In addition, 1-octen-3-ol
301 is produced at high levels by *P. camemberti* during growth, especially in cheese (Abbas and
302 Dobson, 2011), therefore its role in controlling other steps of the *P. camemberti* growth cycle
303 cannot be excluded and should be further studied. Finally, 3-octanone, a methyl-ketone
304 derived from linoleic acid which is also produced by *P. camemberti* (Adda et al., 1989), also
305 deserves further attention since this compound was identified as a conidiogenic compound in
306 *A. nidulans* (Herrero-Garcia et al., 2011).

307 In the last part of the present study, we investigated whether the initial spore inoculum could
308 impact the *P. camemberti* colonization process in model cheeses. We found that estimated
309 biomass by ergosterol measurements was similar regardless of initial inoculum, suggesting
310 that the germination and growth coordination phenomena could also occur in cheese. In
311 addition, low inoculum levels containing 10^2 - 10^3 spores.g⁻¹ of cheese could be sufficient to
312 maximize *P. camemberti* growth. In the cheese industry, the inoculation level of *P.*
313 *camemberti* is empirically determined as a function of the organoleptic properties desired in
314 final product (surface appearance, texture and flavor). Better knowledge of the phenomena
315 governing the *P. camemberti* colonization process could be useful to better control growth
316 and metabolic activities during cheese ripening.

317

318 **Conclusions**

319 In the present study, self-regulation of spore germination by quorum sensing in *P. camemberti*
320 was shown. The volatile nature of the involved compounds was demonstrated and 1-octanol
321 was found to be the main volatile compound produced at high spore density. Its inhibitory
322 effect was verified *in vitro*, showing that 3 mM 1-octanol totally inhibited spore germination

323 while 100 μ M only transiently inhibited this process. This is the first time that spore
324 germination self-inhibition is detected in *P. camemberti* and such knowledge could be useful
325 to better control the ripening process of mould-ripened cheeses.

326

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331

332

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416 **Figure legends**

417 **Fig. 1.** Germination of *Penicillium camemberti* strain A (A) and B (B) conidia at 25°C in malt
418 extract broth as a function of the tested spore density (10^6 spores.mL⁻¹, ■ and 10^8 spores.mL⁻¹,
419 ▲). The arrows represent the time (20h) at which the spore suspensions were diluted to 10^6
420 spores.mL⁻¹. Two biological replicates and three technical replicates were performed for each
421 strain. Error bars represent standard deviations.

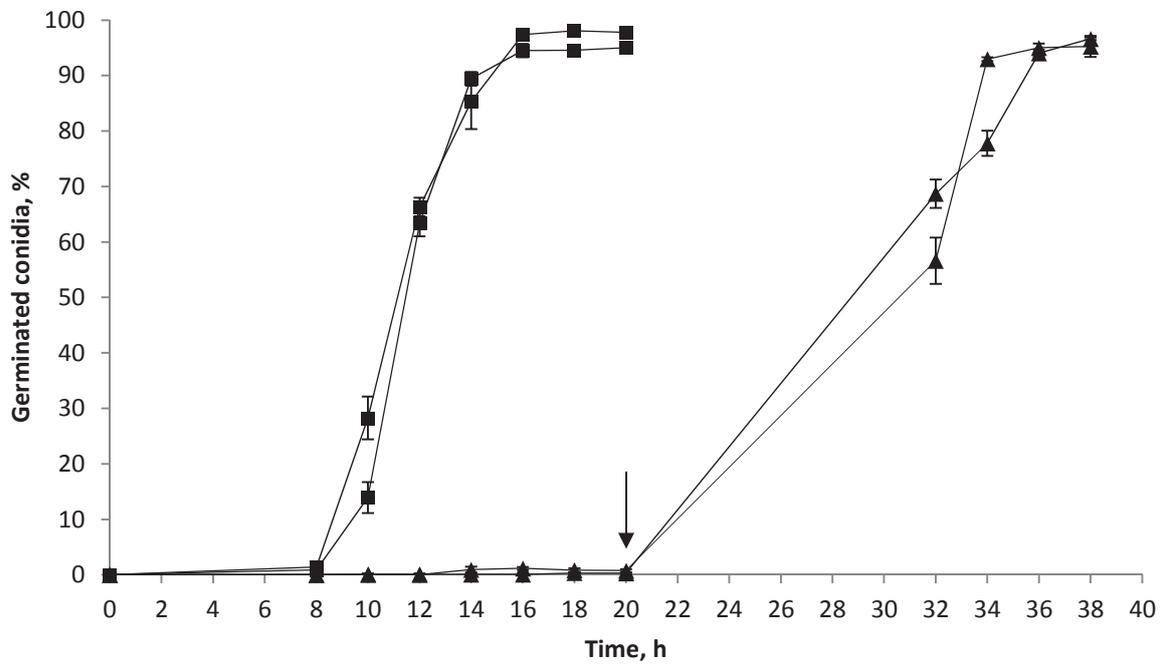
422
423 **Fig. 2.** Germination kinetics (inoculum level of 10^6 spores.mL⁻¹) of *Penicillium camemberti*
424 strain A (■) and B (▲) in filter-sterilized 10^8 spores.mL⁻¹ cultures obtained after 20-h
425 incubation (dotted lines) as compared to germination of strain A (■) and B (▲) in fresh malt
426 extract broth (solid lines). Error bars represent standard deviations.

427
428 **Fig. 3.** Inhibition of spore germination and radial growth of *Penicillium camemberti*
429 cultivated on malt extract agar in the absence (A) or in the presence of a 50 µL at 10^6 (B) or
430 10^8 (C) spores.mL⁻¹ inoculated in the top layer (in the lid) of a Petri dish. CI95%: 95%
431 confidence interval.

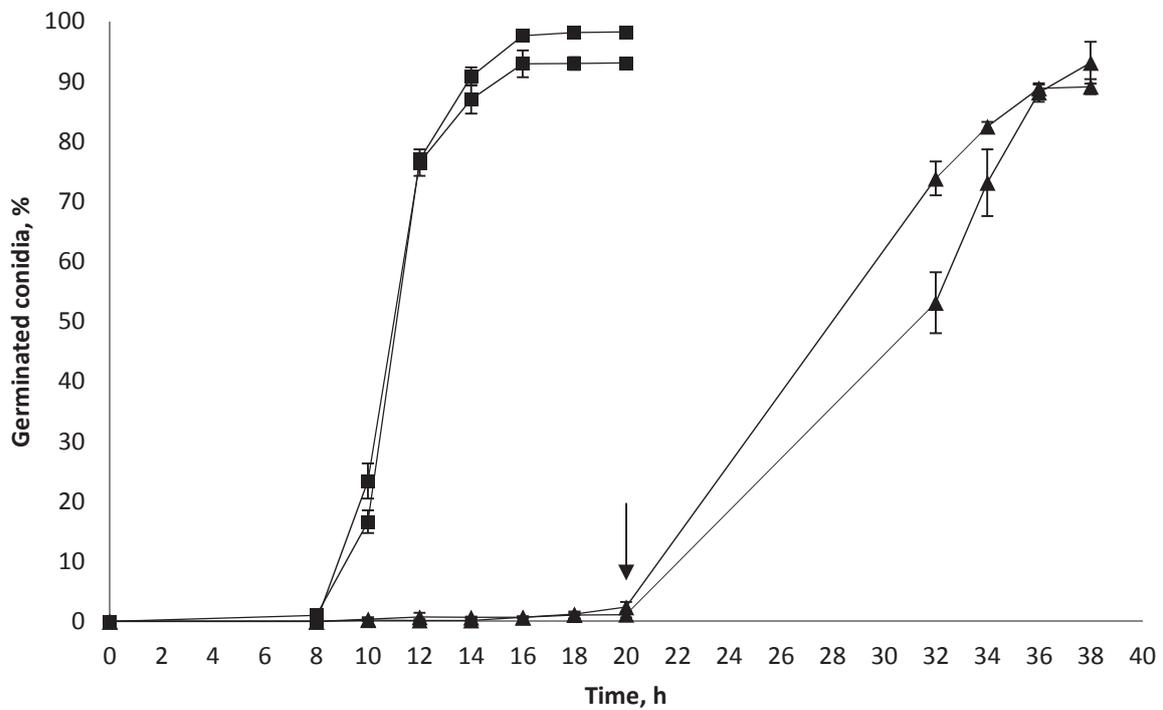
432
433 **Fig. 4.** Chromatograms showing the volatile compounds found in malt extract broth
434 containing 10^8 spores.mL⁻¹ of *Penicillium camemberti* strain A after 20 h incubation at 25°C
435 (A) in comparison with non-inoculated MEB (B) and the mass spectra of the molecule
436 presenting a retention time of 10.997 min (C) in comparison with pure 1-octanol (D).

437
438 **Fig. 5.** Fungal biomass (mean ± 95 % confidence interval) of *Penicillium camemberti* strain A
439 (black bars) and B (grey bars) after 9 days incubation at 12°C in a cheese matrix model as a
440 function of initial spore concentration ranging from 10^2 to 10^6 spores.g⁻¹ cheese.

441

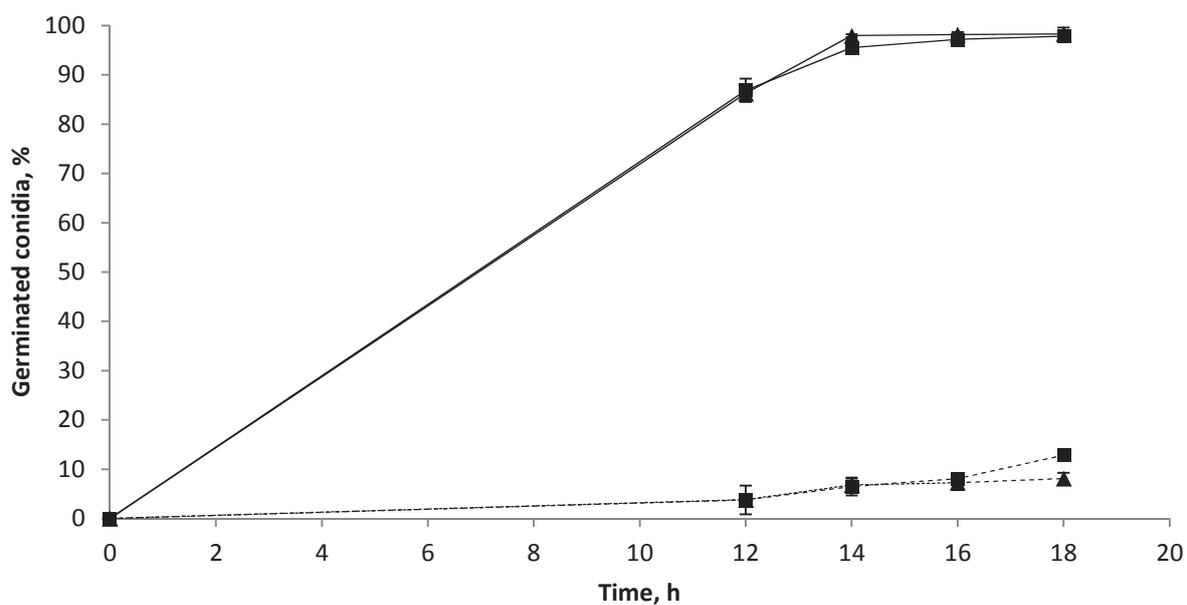
442 **Fig. 1.**443 **A**

444

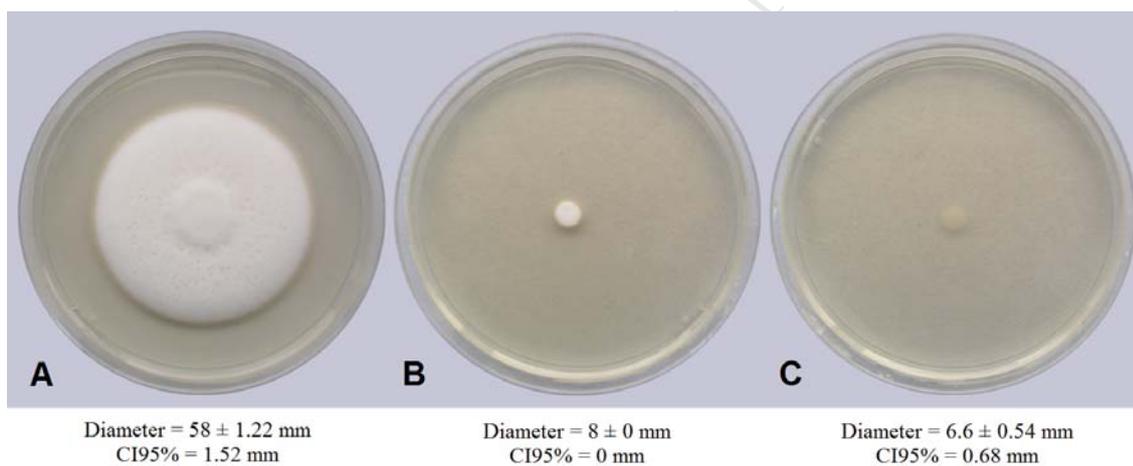
445 **B**

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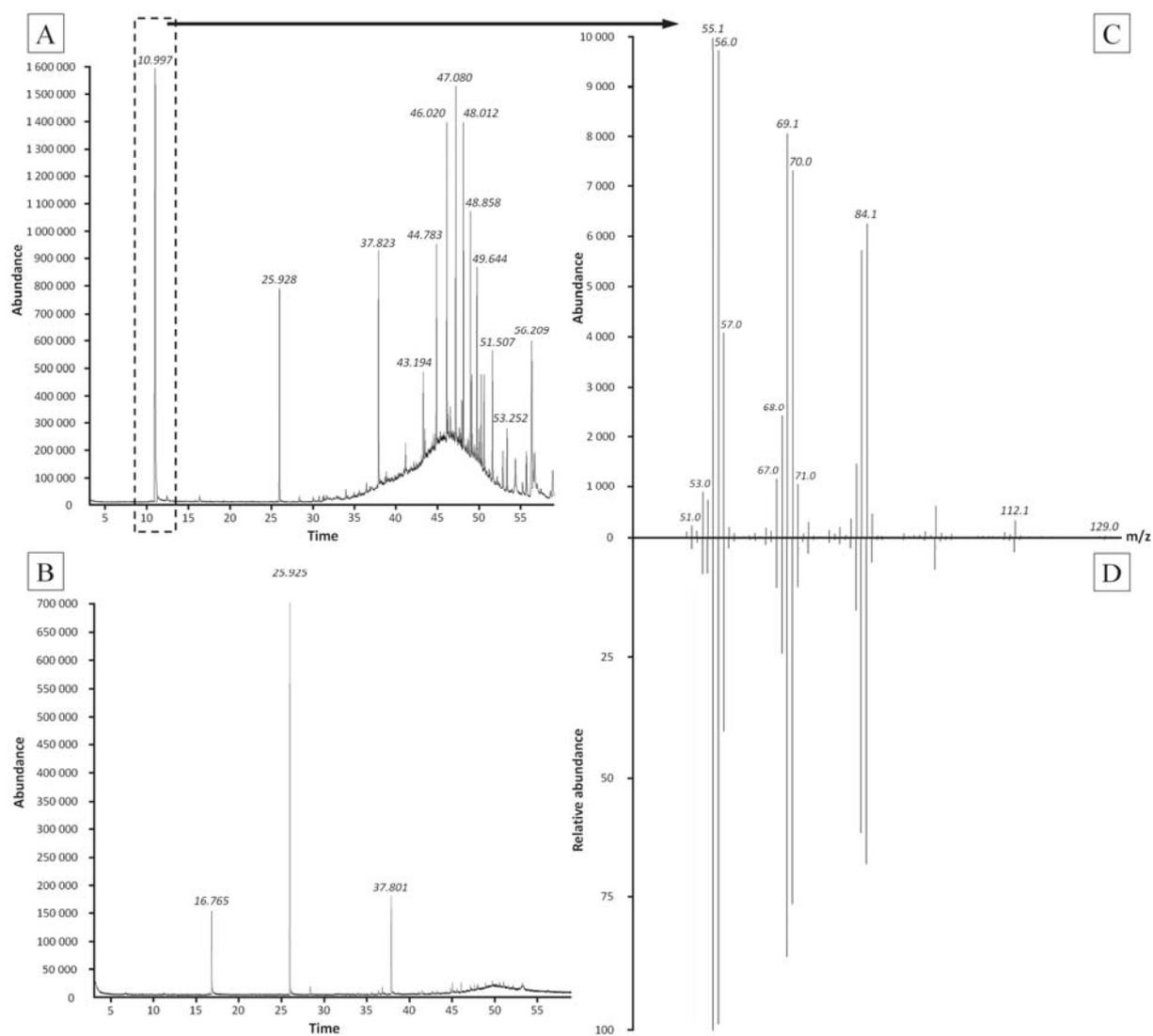
448 **Fig. 2.**

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450 **Fig. 3.**

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453 **Fig. 4.**

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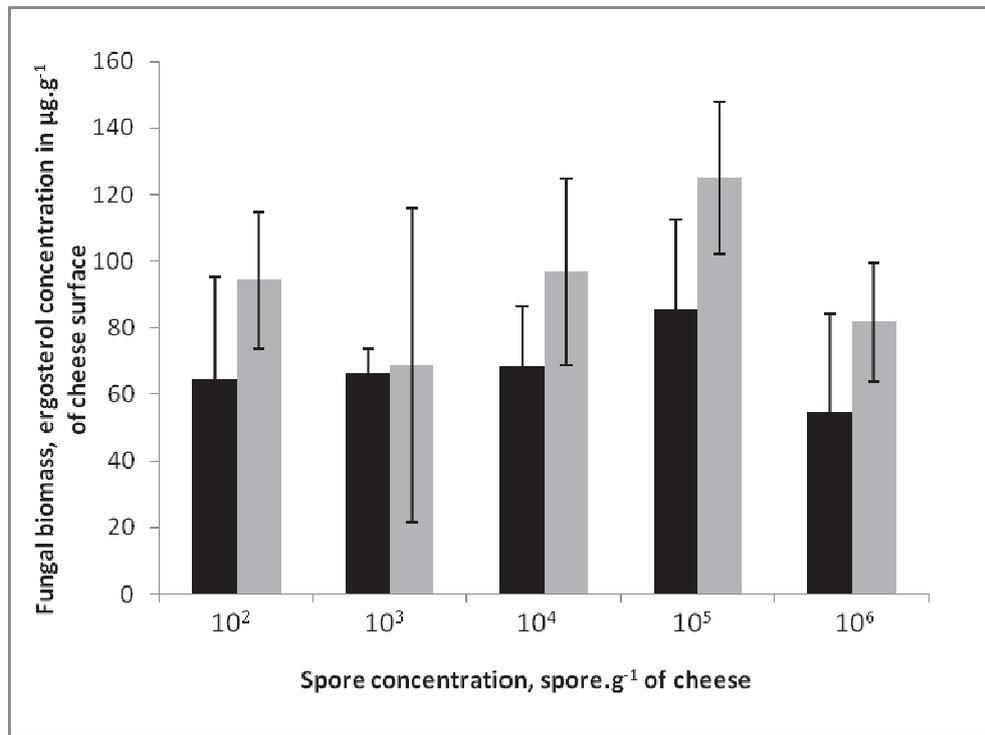
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465 **Fig. 5.**

466

Highlights:

- Spore germination in *Penicillium camemberti* is self-regulated by quorum sensing.
- Quorum sensing is observed when spore densities are high.
- Compounds involved in quorum sensing are volatile.
- 1-octanol was found to be the main volatile compound produced at high-spore density.