

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

Guillaume Gillot, Nicolas Decourcelle, Gaëlle Dauer, Georges Barbier,  
Emmanuel Coton, David Delmail, Jérôme Mounier

► **To cite this version:**

Guillaume Gillot, Nicolas Decourcelle, Gaëlle Dauer, Georges Barbier, Emmanuel Coton, et al.. 1-octanol, a self-inhibitor of spore germination in *Penicillium camemberti*. Food Microbiology, Elsevier, 2016, 57, pp.1-7. 10.1016/j.fm.2015.12.008 . hal-01254795

**HAL Id: hal-01254795**

**<https://hal-univ-rennes1.archives-ouvertes.fr/hal-01254795>**

Submitted on 21 Apr 2016

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

3  
4 Guillaume Gillot<sup>1</sup>, Nicolas Decourcelle<sup>2</sup>, Gaëlle Dauer<sup>2</sup>, Georges Barbier<sup>1</sup>, Emmanuel Coton<sup>1</sup>,  
5 David Delmail<sup>3</sup>, Jérôme Mounier<sup>1\*</sup>.

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

9 <sup>2</sup> *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 <sup>3</sup> *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](mailto:jerome.mounier@univ-brest.fr)

23

**Abstract**

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

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

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

## 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<sup>T</sup> 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  $-80^{\circ}\text{C}$  in 10 % glycerol (v/v), and  
101 incubating tubes for 7 days at  $25^{\circ}\text{C}$ . Strains were then sub-cultured on slant PDA and  
102 incubated at  $25^{\circ}\text{C}$  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  $25^{\circ}\text{C}$ , 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  $4^{\circ}\text{C}$  (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<sup>-1</sup>.

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<sup>-1</sup>) were mixed with 0.75 mL MEB 2X to  
120 obtain a final spore concentration of  $10^8$  or  $10^6$  spores.mL<sup>-1</sup>. Suspensions were then incubated  
121 horizontally in a rotary shaker set to  $25^{\circ}\text{C}$  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<sup>-1</sup> 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<sup>-1</sup> 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  $\mu$ L of a  $10^6$  or  $10^8$   
138 spores.mL<sup>-1</sup> suspension while 5  $\mu$ L of a  $10^7$  spores.mL<sup>-1</sup> 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 Na<sub>2</sub>SO<sub>4</sub> 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<sup>-1</sup> between 50°C and  
157 200°C then 10°C.min<sup>-1</sup> 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<sup>-1</sup>; mass acquisition 50-300 uma. The vector gas was H<sub>2</sub> at a  
161 1.5 mL.min<sup>-1</sup> 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 10<sup>6</sup> spore.mL<sup>-1</sup> 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<sup>-1</sup>. 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<sup>-1</sup> 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<sup>-1</sup> 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 10<sup>6</sup> spores.mL<sup>-1</sup> presented higher germination percentages compared to 10<sup>8</sup>  
210 spores.mL<sup>-1</sup> cultures. Indeed, after 20h incubation, >90 % and <3% spores had germinated at  
211 10<sup>6</sup> versus 10<sup>8</sup> spores.mL<sup>-1</sup>. Similar results were also obtained on *P. camemberti* strains  
212 UBOCC-A-101392, UBOCC-A-108097 and CBS 299.48<sup>T</sup> (data not shown). To determine  
213 whether germination inhibition was the consequence of higher spore density, 10<sup>8</sup> spores.mL<sup>-1</sup>  
214 cultures obtained after 20 h incubation were diluted to 10<sup>6</sup> spores.mL<sup>-1</sup> 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 10<sup>8</sup>  
218 spores.mL<sup>-1</sup> culture filtrates were inoculated with a new spore suspension (10<sup>6</sup> spores.mL<sup>-1</sup>)  
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 10<sup>8</sup> spores.mL<sup>-1</sup>.

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  $\mu\text{L}$  of a spore suspension containing  $10^6$  (Fig. 3B) or  $10^8$  (Fig.  
230 3C) spores. $\text{mL}^{-1}$ , the latter having the strongest effect. Indeed, mean radial growth rates were  
231 4.14, 0.57 and 0.47  $\text{mm.d}^{-1}$  for the control and the lid agar inoculated with  $10^6$  and  $10^8$   
232 spores. $\text{mL}^{-1}$ , 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. $\text{mL}^{-1}$  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  $\mu$ 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<sup>-1</sup> 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<sup>-1</sup> 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  $\mu$ 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

## 327 Acknowledgements

328 The authors are thankful to CBB développement and the Région Bretagne for their financial  
329 support. We thank Danielle Arzur for technical assistance and Doctor Monika Coton for  
330 English revision.

331

332

## 333 References

- 334 Abbas, A., Dobson, A.D.W., 2011. Yeasts and Molds: *Penicillium camemberti*, in: Fuquay,  
335 J.W. (Ed.), Encyclopedia of Dairy Sciences. Academic Press, San Diego, pp. 776–779.
- 336 Adda, J., Dekimpe, J., Vassal, L., Spinnler, H.E., 1989. Production de styrène par *Penicillium*  
337 *camemberti* Thom. Le Lait 69, 115–120. doi:10.1051/lait:198928
- 338 Bacon, C.W., Sussman, A.S., Paul, A.G., 1973. Identification of a self-inhibitor from spores  
339 of *Dictyostelium discoideum*. J. Bacteriol. 113, 1061–1063.
- 340 Barrios-González, J., Martínez, C., Aguilera, A., Raimbault, M., 1989. Germination of  
341 concentrated suspensions of spores from *Aspergillus niger*. Biotechnol. Lett. 11, 551–  
342 554. doi:10.1007/BF01040034
- 343 Beresford, T., Williams, A., 2004. The microbiology of cheese ripening, in: Fox, P.F.,  
344 McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics  
345 and Microbiology. Elsevier Academic Press, London, pp. 287–317.
- 346 Chitarra, G.S., Abee, T., Rombouts, F.M., Dijksterhuis, J., 2005. 1-Octen-3-ol inhibits conidia  
347 germination of *Penicillium paneum* despite of mild effects on membrane permeability,  
348 respiration, intracellular pH, and changes the protein composition. FEMS Microbiol.  
349 Ecol. 54, 67–75. doi:10.1016/j.femsec.2005.02.013
- 350 Chitarra, G.S., Abee, T., Rombouts, F.M., Posthumus, M.A., Dijksterhuis, J., 2004.  
351 Germination of *Penicillium paneum* conidia is regulated by 1-Octen-3-ol, a volatile  
352 self-inhibitor. Appl. Environ. Microbiol. 70, 2823–2829.  
353 doi:10.1128/AEM.70.5.2823-2829.2004
- 354 Combet, E., Eastwood, D.C., Burton, K.S., Combet, E., Henderson, J., Henderson, J.,  
355 Combet, E., 2006. Eight-carbon volatiles in mushrooms and fungi: properties,  
356 analysis, and biosynthesis. Mycoscience 47, 317–326. doi:10.1007/S10267-006-0318-  
357 4
- 358 Dantigny, P., Bensoussan, M., Vasseur, V., Lebrihi, A., Buchet, C., Ismaili-Alaoui, M.,  
359 Devlieghere, F., Roussos, S., 2006. Standardisation of methods for assessing mould

- 360 germination: a workshop report. *Int. J. Food Microbiol.* 108, 286–291.  
361 doi:10.1016/j.ijfoodmicro.2005.12.005
- 362 Dijksterhuis, J., Samson, R.A., 2002. Food and crop spoilage on storage, in: Kempken, P.D.F.  
363 (Ed.), *Agricultural Applications, The Mycota*. Springer Berlin Heidelberg, pp. 39–52.
- 364 Felkner, I.C., Wyss, O., 1964. A substance produced by competent *Bacillus cereus* 569 cells  
365 that affects transformability. *Biochem. Biophys. Res. Commun.* 16, 94–99.  
366 doi:10.1016/0006-291X(64)90217-7
- 367 Fuqua, W.C., Winans, S.C., Greenberg, E.P., 1994. Quorum sensing in bacteria: the LuxR-  
368 LuxI family of cell density-responsive transcriptional regulators. *J. Bacteriol.* 176,  
369 269–275.
- 370 Garrett, M.K., Robinson, P.M., 1969. A stable inhibitor of spore germination produced by  
371 fungi. *Arch. Für Mikrobiol.* 67, 370–377. doi:10.1007/BF00412583
- 372 Herrero-Garcia, E., Garzia, A., Cordobés, S., Espeso, E.A., Ugalde, U., 2011. 8-Carbon  
373 oxylipins inhibit germination and growth, and stimulate aerial conidiation in  
374 *Aspergillus nidulans*. *Fungal Biol.* 115, 393–400. doi:10.1016/j.funbio.2011.02.005
- 375 Hobot, J.A., Gull, K., 1980. The identification of a self-inhibitor from *Syncephalastrum*  
376 *racemosum* and its effect upon sporangiospore germination. *Antonie Van*  
377 *Leeuwenhoek* 46, 435–441.
- 378 Hogan, D.A., 2006. Talking to themselves: autoregulation and quorum sensing in fungi.  
379 *Eukaryot. Cell* 5, 613–619. doi:10.1128/EC.5.4.613-619.2006
- 380 Husson, F., Thomas, M., Kermasha, S., Belin, J.-M., 2002. Effect of linoleic acid induction on  
381 the production of 1-octen-3-ol by the lipoxygenase and hydroperoxide lyase activities  
382 of *Penicillium camemberti*. *J. Mol. Catal. B Enzym., Proceedings of the 5th*.  
383 *International Symposium on Biocatalysis and Biotransformations* 19–20, 363–369.  
384 doi:10.1016/S1381-1177(02)00187-X
- 385 Lax, A.R., Templeton, G.E., Meyer, W.L., 1985. Isolation, purification, and biological  
386 activity of a self-inhibitor from conidia of *Colletotrichum gloeosporioides*.  
387 *Phytopathol. Biochem.*
- 388 Le Dréan, G., Mounier, J., Vasseur, V., Arzur, D., Habrylo, O., Barbier, G., 2010.  
389 Quantification of *Penicillium camemberti* and *P. roqueforti* mycelium by real-time  
390 PCR to assess their growth dynamics during ripening cheese. *Int. J. Food Microbiol.*  
391 138, 100–107. doi:10.1016/j.ijfoodmicro.2009.12.013
- 392 Leite, B., Nicholson, R.L., 1992. Mycosporine-alanine: A self-inhibitor of germination from  
393 the conidial mucilage of *Colletotrichum graminicola*. *Exp. Mycol.* 16, 76–86.  
394 doi:10.1016/0147-5975(92)90043-Q
- 395 Lingappa, B.T., Lingappa, Y., Bell, E., 1973. A self-inhibitor of protein synthesis in the  
396 conidia of *Glomerella cingulata*. *Arch. Für Mikrobiol.* 94, 97–107.  
397 doi:10.1007/BF00416685
- 398 Macko, V., Staples, R.C., Renwick, J.A.A., Pirone, J., 1972. Germination self-inhibitors of  
399 rust uredospores. *Physiol. Plant Pathol.* 2, 347–355. doi:10.1016/0048-  
400 4059(72)90060-4
- 401 Maga, J.A., 1981. Mushroom flavor. *J. Agric. Food Chem.* 29, 1–4. doi:10.1021/jf00103a001
- 402 Nealson, K.H., Hastings, J.W., 1979. Bacterial bioluminescence: its control and ecological  
403 significance. *Microbiol. Rev.* 43, 496–518.
- 404 Raper, K.B., Thom, C., 1949. *A manual of the Penicillia*. Williams and Wilkens Co.,  
405 Baltimore.
- 406 Tomasz, A., Hotchkiss, R.D., 1964. Regulation of the transformability of pneumococcal  
407 cultures by macromolecular cell products. *Proc. Natl. Acad. Sci. U. S. A.* 51, 480–487.



- 408 Tressl, R., Bahri, D., Engel, K.H., 1982. Formation of eight-carbon and ten-carbon  
409 components in mushrooms (*Agaricus campestris*). J. Agric. Food Chem. 30, 89–93.  
410 doi:10.1021/jf00109a019
- 411 Venkateshwarlu, G., Chandradana, M.V., Tewari, R.P., 1999. Volatile flavour components  
412 of some edible mushrooms (Basidiomycetes). Flavour Fragr. J. 14, 191–194.  
413 doi:10.1002/(SICI)1099-1026(199905/06)14:3<191::AID-FFJ810>3.0.CO;2-7  
414  
415

ACCEPTED MANUSCRIPT

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<sup>-1</sup>, ■ and  $10^8$  spores.mL<sup>-1</sup>,  
419 ▲). The arrows represent the time (20h) at which the spore suspensions were diluted to  $10^6$   
420 spores.mL<sup>-1</sup>. Two biological replicates and three technical replicates were performed for each  
421 strain. Error bars represent standard deviations.

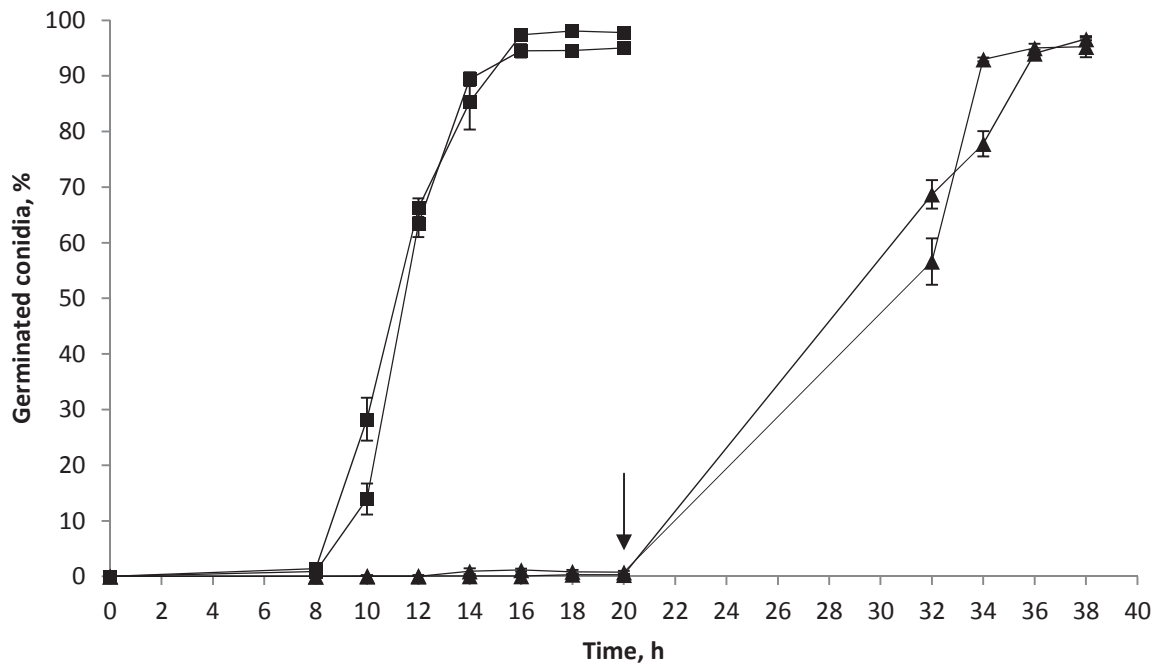
422  
423 **Fig. 2.** Germination kinetics (inoculum level of  $10^6$  spores.mL<sup>-1</sup>) of *Penicillium camemberti*  
424 strain A (■) and B (▲) in filter-sterilized  $10^8$  spores.mL<sup>-1</sup> cultures obtained after 20-h  
425 incubation (dotted lines) as compared to germination of strain 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<sup>-1</sup> inoculated in the top layer (in the lid) of a Petri dish. CI95%: 95%  
431 confidence interval.

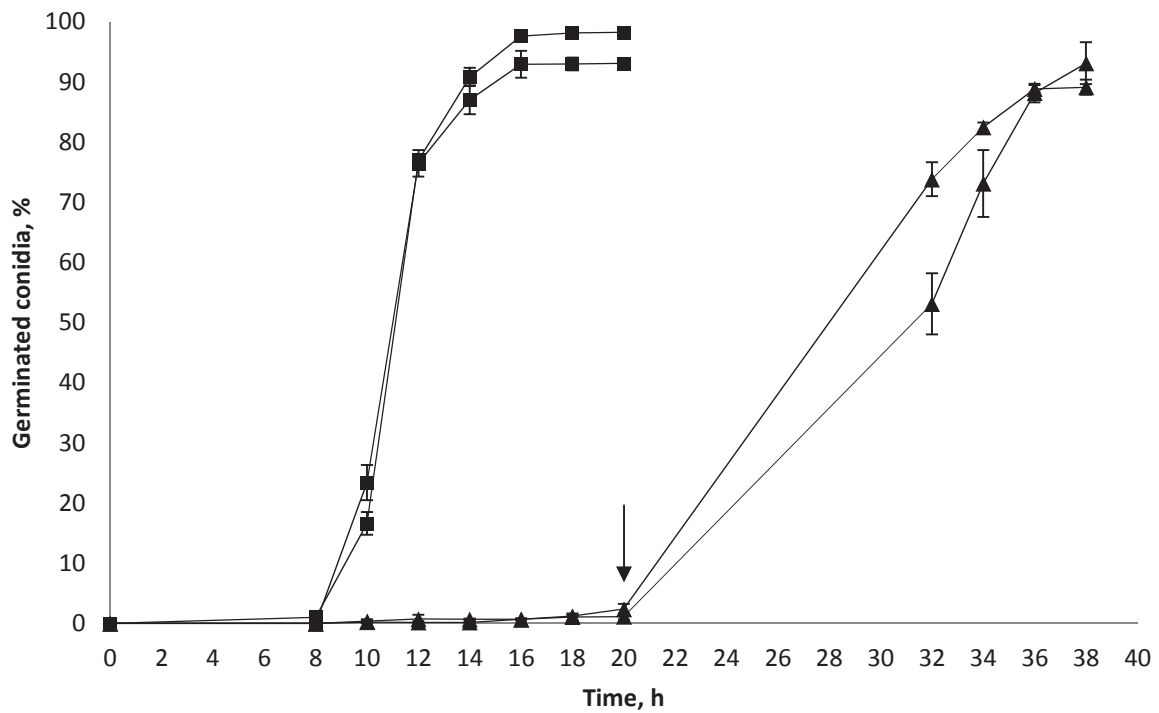
432  
433 **Fig. 4.** Chromatograms showing the volatile compounds found in malt extract broth  
434 containing  $10^8$  spores.mL<sup>-1</sup> of *Penicillium camemberti* strain A after 20 h incubation at 25°C  
435 (A) in comparison with non-inoculated MEB (B) and the mass spectra of the molecule  
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<sup>-1</sup> cheese.

441

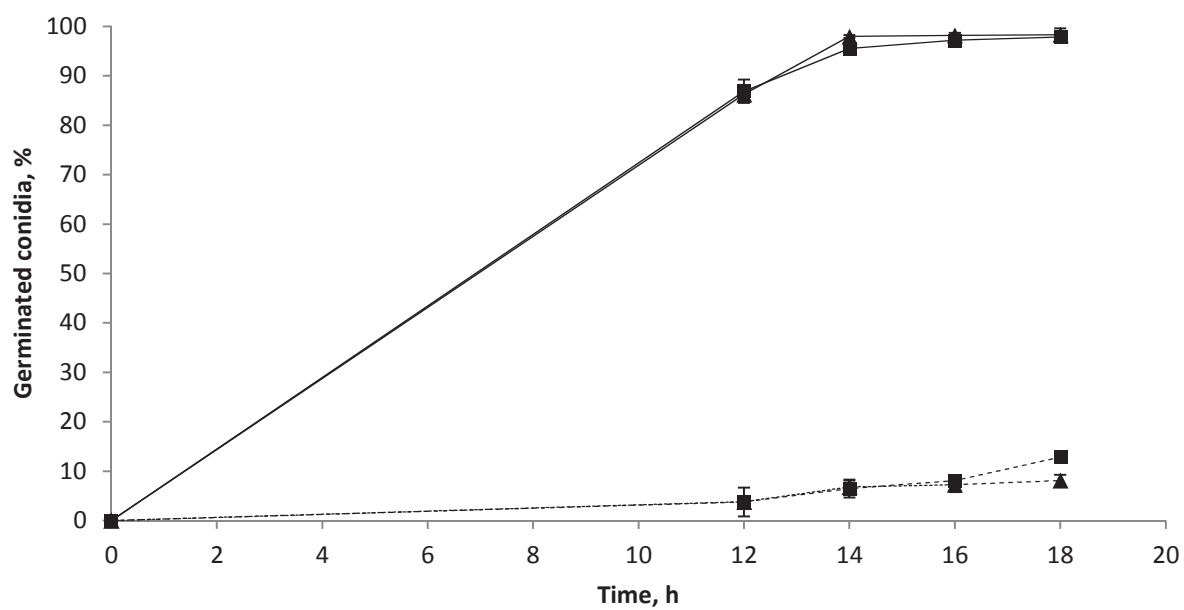
442 **Fig. 1.**443 **A**

444

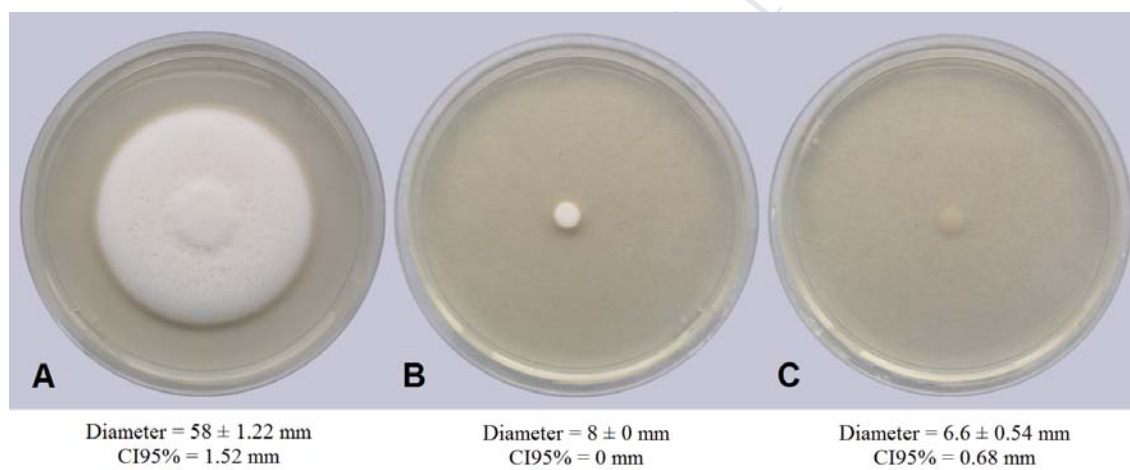
445 **B**

446

447

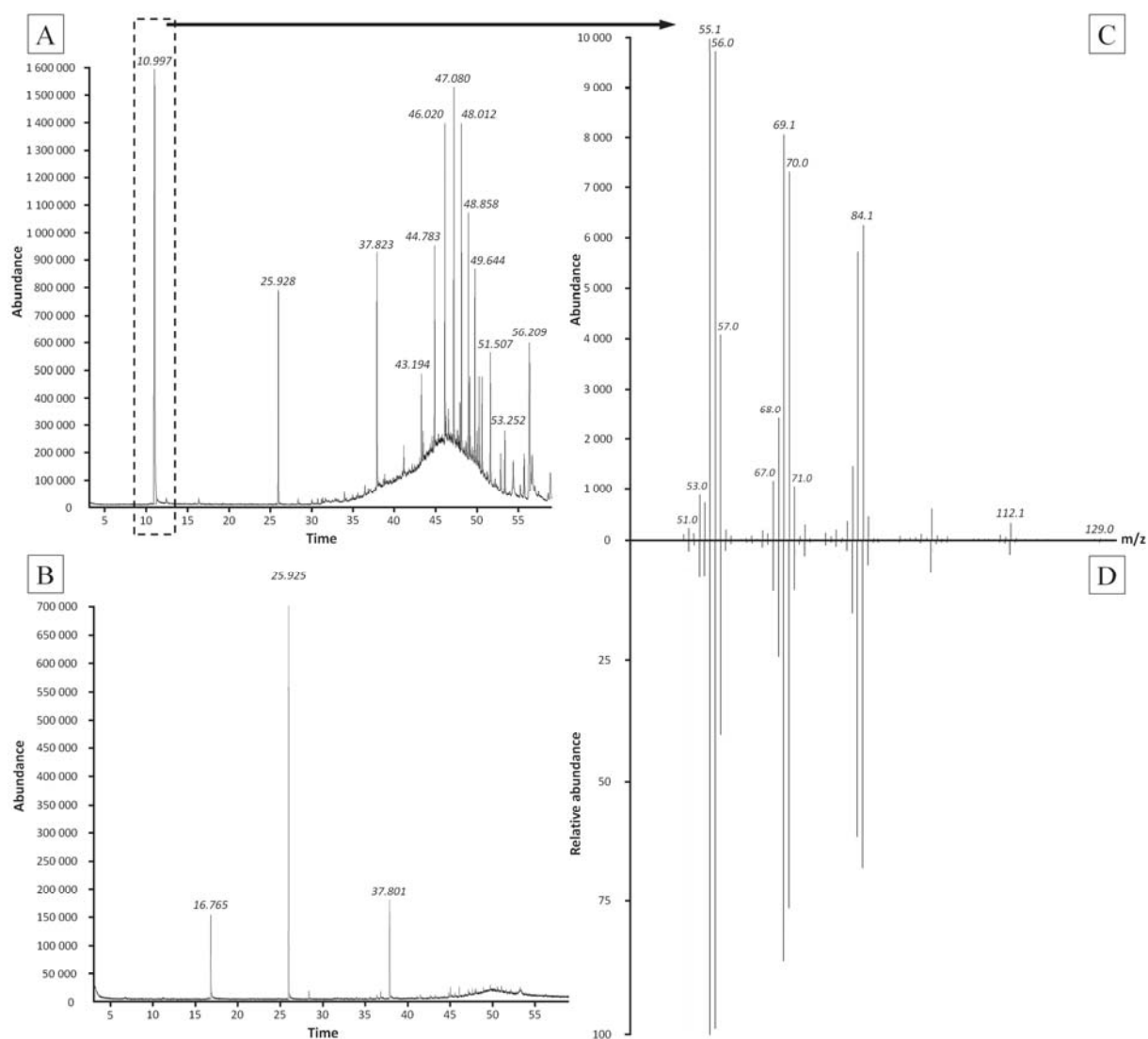
448 **Fig. 2.**

449

450 **Fig. 3.**

451

452

453 **Fig. 4.**

454

455

456

457

458

459

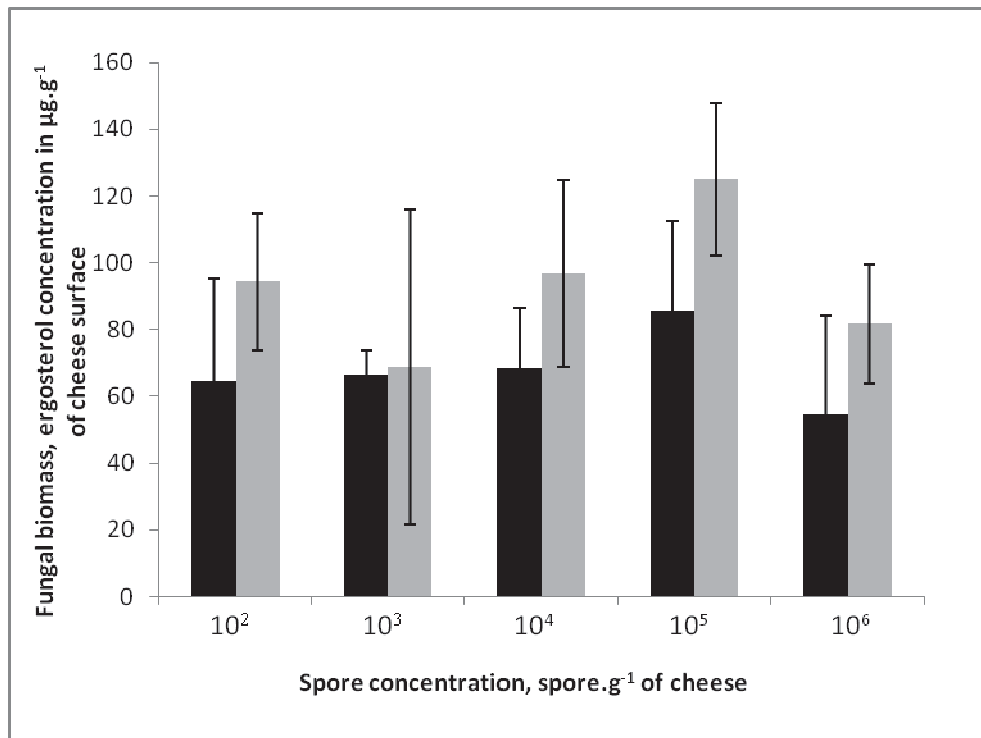
460

461

462

463

464

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.