

Development of antifungal ingredients for dairy products: From in vitro screening to pilot scale application

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1 Development of antifungal ingredients for dairy products: from *in vitro* screening to

- 2 pilot scale application.
- 3
- 4 Lucille Garnier^{1,2}, Jérôme Mounier¹, Sébastien Lê³, Audrey Pawtowski¹, Nicolas Pinon²,
 5 Bénédicte Camier², Manon Chatel², Gilles Garric², Anne Thierry², Emmanuel Coton¹ and
 6 Florence Valence²*.
- 7
- 8 ¹Université de Brest, EA 3882, Laboratoire Universitaire de Biodiversité et Ecologie
- 9 Microbienne, ESIAB, Technopôle Brest-Iroise, 29280 Plouzané, France.
- 10 ² UMR1253 Science et Technologie du Lait et de l'OEuf, INRA, Agrocampus Ouest, 65 rue
- 11 de Saint Brieuc, 35000 Rennes, France
- ³ Applied Mathematics Department, Agrocampus Ouest, 65 rue de Saint-Brieuc, 35042
- 13 Rennes cedex, France
- 14
- 15 Lucille Garnier: lucille.garnier@univ-brest.fr
- 16 Jérôme Mounier: jerome.mounier@univ-brest.fr
- 17 Sebastien Lê: sebastien.le@agrocampus-ouest.fr
- 18 Audrey Pawtowski: audrey.pawtowski@univ-brest.fr
- 19 Nicolas Pinon: nicolas.pinon@inra.fr
- 20 Bénédicte Camier: benedicte.camier@inra.fr
- 21 Manon Chatel: manon.chatel@inra.fr
- 22 Gilles Garric: gilles.garric@inra.fr
- 23 Anne Thierry: anne.thierry@inra.fr
- 24 Emmanuel Coton: emmanuel.coton@univ-brest.fr
- 25

- 26 ***Corresponding author:** Dr. Florence Valence
- 27 INRA UMR 1253 Science et Technologie du Lait et de l'œuf
- 28 F-35042, Rennes cedex
- 29 Tel: +33 (0)2 23 43 53 23
- 30 Fax: +33 (0)2 23 48 53 50
- 31 E-mail: florence.valence-bertel@inra.fr

32 Abstract

33 Biopreservation represents a complementary approach to traditional hurdle technologies for reducing microbial contaminants (pathogens and spoilers) in food. In 34 35 the dairy industry that is concerned by fungal spoilage, biopreservation can also be an alternative to preservatives currently used (e.g. natamycin, potassium sorbate). The aim 36 37 of this study was to develop antifungal fermentates derived from two dairy substrates 38 using a sequential approach including an *in vitro* screening followed by an *in situ* 39 validation. The *in vitro* screening of the antifungal activity of fermentates derivating from 430 lactic acid bacteria (LAB) (23 species), 70 propionibacteria (4 species) and 198 40 41 fungi (87 species) was performed against four major spoilage fungi (Penicillium 42 commune, Mucor racemosus, Galactomyces geotrichum and Yarrowia lipolytica) using a 43 cheese-mimicking model. The most active fermentates were obtained from Lactobacillus 44 brevis, Lactobacillus buchneri, Lactobacillus casei/paracasei and Lactobacillus plantarum 45 among the tested LAB, Propionibacterium jensenii among propionibacteria, and Mucor 46 lanceolatus among the tested fungi. Then, for the 11 most active fermentates, culture 47 conditions were optimized by varying incubation time and temperature in order to enhance their antifungal activity. Finally, the antifungal activity of 3 fermentates of 48 49 interest obtained from Lactobacillus rhamnosus CIRM-BIA1952, P. jensenii CIRM-50 BIA1774 and M. lanceolatus UBOCC-A-109193 were evaluated in real dairy products 51 (sour cream and semi-hard cheese) at a pilot-scale using challenge and durability tests. 52 In parallel, the impact of these ingredients on organoleptic properties of the obtained 53 products was also assessed. In semi-hard cheese, application of the selected fermentates 54 on the cheese surface delayed the growth of spoilage molds for up to 21 days, without 55 any effect on organoleptic properties, P. jensenii CIRM-BIA1774 fermentate being the most active. In sour cream, incorporation of the latter fermentate at 2 or 5% yielded a 56

57 high antifungal activity but was detrimental to the product organoleptic properties. 58 Determination of the concentration limit, compatible with product acceptability, showed 59 that incorporation of this fermentate at 0.4% prevented growth of fungal contaminants in durability tests but had a more limited effect against M. racemosus and P. commune 60 in challenge tests. To our knowledge, this is the first time that the workflow followed in 61 this study, from *in vitro* screening using dairy matrix to scale-up in cheese and sour 62 cream, is applied for production of natural ingredients relying on a large microbial 63 64 diversity in terms of species and strains. This approach allowed obtaining several antifungal fermentates which are promising candidates for dairy products 65 biopreservation. 66

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Keywords: fungal spoilage, biopreservation, lactic acid bacteria, propionibacteria, fungi.
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70 Introduction

71 Fungal spoilage of dairy products is responsible for significant food waste and economic 72 losses (Pitt and Hocking, 2009). Dairy products, despite their acidic pH and high content in 73 organic acids, may provide a favorable environment for the development of undesirable 74 microorganisms including fungi. Fungal spoilage is usually easy to detect due to the presence 75 of colonies or thalli at the surface of the product. Nonetheless, fungal spoilage may also be 76 non-visible resulting in flavor or texture alteration via fungal metabolism (Ledenbach and 77 Marshall, 2010), thus preventing product consumption. According to the literature, species 78 belonging to the Penicillium, Mucor, Cladosporium,, Candida, Galactomyces and Yarrowia genera have been identified as the most common contaminating molds and yeasts, 79 80 respectively (Pitt and Hocking, 2009; Garnier et al., 2017a).

81 In order to avoid or delay fungal spoilage, and hence extend product shelf life, prevention and control methods are currently used by industrials. Prevention methods applied in the dairy 82 83 industry include good manufacturing and hygiene practices, implementation of Hazard 84 Analysis Critical Control Point (HACCP) system, and use of air filtration or packaging in 85 aseptic conditions (Garnier et al., 2017a). Control methods can involve the use of heat 86 treatment, modified atmosphere packaging or fermentation with beneficial microorganisms (Garnier et al., 2017a; Leyva Salas et al., 2017; Nguyen Van Long et al., 2016; Sakkas et al., 87 88 2014; Bourdichon et al., 2012). In specific dairy products such as sour cream or semi-hard 89 cheese, the addition of defined chemical preservatives with antifungal properties (e.g. 90 natamycin or organic acids and their salts) is permitted depending on the regulation in place 91 (Leyva Salas et al., 2017). However, there is a strong societal demand for "preservative-free" 92 food products since consumers are looking for more "natural" (i.e. without chemical 93 preservatives) and less heavily processed foods. Therefore, industrial groups are looking for

94 efficient solutions to replace chemical preservatives while maintaining the current shelf-life of
95 food products (Varsha et al., 2016).

In this context, biopreservation, defined as the use of natural or added microbiota and/or their 96 97 antimicrobial compounds to extend food shelf-life and increase food safety (Stiles, 1996), 98 could represent a complementary approach to reduce fungal spoilage and when applicable, an 99 alternative to chemical preservatives. The use of lactic acid bacteria (LAB) and 100 propionibacteria and/or their metabolites is particularly interesting for reducing fungal 101 spoilage in dairy products (Leyva Salas et al., 2017). Indeed, LAB and propionibacteria are 102 naturally present in many foods and have a long history of safe use in fermented foods 103 (Bourdichon et al., 2012). They produce a wide range of antifungal compounds including 104 organic acids such as acetic, lactic, phenyllactic, propionic and fatty acids as well as cyclic 105 dipeptides or proteinaceous compounds (Crowley et al., 2013, Schnürer and Magnusson, 106 2005, Lavermicocca et al., 2000). Fungal inhibition generally results from the additive and/or 107 synergistic activities of several of these compounds (Varsha et al., 2016). In addition to LAB 108 and propionibacteria, some fungi can also produce antifungal compounds such as alcohols, 109 acids, esters, and proteinaceous compounds (Delgado et al., 2016, Leyva-Salas et al., 2017), 110 and their utilization could also be compatible with some dairy technologies.

Commercial antifungal solutions for dairy products are currently available on the market. All of them involve LAB and/or propionibacteria in single or culture combinations. However, their efficiency varies according to the dairy technologies in which they are used (Leyva-Salas et al., 2017; Garnier et al., 2017b). These microorganisms can be used as adjunct cultures (i.e. co-inoculated with the starter culture during product manufacturing) or to produce fermentates (e.g. resulting from dairy substrate fermentation) added to food as an ingredient (Varsha et al., 2016; Garnier et al. 2017b). While there is an increasing number of studies showing the *in vitro* antifungal activity of selected microorganisms and/or their metabolites for application in dairy products or other foods (Leyva-Salas et al., 2017), very few have dealt with the efficiency of such antifungal ingredients in real products and their potential impact on product organoleptic properties (Lynch et al., 2014, Delavenne et al., 2015). Moreover, to our best knowledge, no work on the development of a biopreservation ingredient to replace natamycin (E235 in the European Union) for cheese surface-treatment has been reported yet in the literature.

The aim of this study was to develop dairy antifungal biopreservation ingredients obtained by fermentation, by selected microorganisms, of 2 dairy substrates (low-heat milk -LH- and milk permeate -UF-). In this framework, a sequential approach based on *in vitro* screening and *in situ* validation at a pilot scale on actual dairy products, namely sour cream and semi-hardcheese, was used.

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131 Materials and methods

132 2.1. Fermentate production

133 Two different dairy substrates, UF and LH, were used to prepare fermentates. The UF 134 substrate consisted of an ultrafiltration milk permeate (T.I.A., Bollene, France) supplemented 135 with 10 g/L yeast extract (Biokar Diagnosis) and 0.5 % litmus, and filtrated at 0.22 µm 136 (VWR, Radnor, USA). The LH substrate was obtained as described by Garnier et al. (2018). 137 Both substrates were then individually inoculated by 430 LAB, 70 propionibacteria or 198 138 fungal strains obtained from the Laboratoire Universitaire de Biodiversité et Ecologie 139 Microbienne (LUBEM, Plouzané, France), the Université de Bretagne Occidentale Culture 140 Collection (UBOCC, Plouzané, France, www.univ-brest.fr/ubocc) and the CIRM-BIA (Centre 141 International de Ressources Microbienne-Bactéries d'Intérêt Alimentaire, Rennes, 142 www.collection-cirmbia.fr) culture collection (Supplementary Table S1). For LAB and

143 propionibacteria strains, respectively, LH and UF substrates were inoculated with bacterial 144 MRS and YEL broth pre-cultures (Difco, Le Pont de Chaix, France) according to Garnier et 145 al. (2018), except for Lactobacillus amylovorus, Lactobacillus gasseri, Lactobacillus 146 acidophilus, Lactobacillus delbrueckii and Lactobacillus fermentum strains which were pre-147 cultured and incubated at 37°C. Lactobacillus sanfranciscensis and Lactococcus lactis strains 148 were pre-cultured in MRS and M17 supplemented with 20 g/L maltose, respectively (Difco, 149 Le Pont de Claix, France). For yeasts, LH and UF media were inoculated (1% v/v) after 2 pre-150 cultures (24 h at 25°C, 120 rpm agitation speed) in Yeast Glucose Chloramphenicol (YGC) 151 broth (Difco, Le Pont de Claix, France), and incubated for 48 h in the same conditions. Concerning molds, they were first cultivated on Potato Dextrose Agar (PDA) slant for 7 d at 152 25°C. After spore recovery with 0.01 % Tween 80 and enumeration using a Malassez cell, LH 153 and UF media were inoculated at 10^4 spores/ml and incubated for 96 h at 25°C on a rotary 154 155 shaker at 120 rpm. Yeast and mold fermentates were centrifuged three times (8000g at 156 15 min), the cell pellets were discarded and the final supernatant was filter-sterilized at 157 0.45 µm (VWR, Radnor, USA). Fermentates were then stored at -80°C until use. For pilot 158 scale applications, fermentates were produced in 2 to 5-L flasks. Bacterial fermentates were 159 lyophilized (Virtis, Fisher, France, final residual water < 2% after freeze drying under 160 vacuum) while fungal fermentates were prepared as previously described.

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2.2. In vitro screening of antifungal activity

The antifungal activity of the obtained fermentates against *Mucor racemosus* UBOCC-A-164 116002, *Penicillium commune* UBOCC-A-116003, *Galactomyces geotrichum* UBOCC-A-165 216001 and *Yarrowia lipolytica* UBOCC-A-216006, isolated from spoiled dairy products and 166 representative of the most common dairy product spoilers (Garnier et al., 2017b), was 167 evaluated *in vitro* using a high-throughput screening method in a cheese-mimicking model as168 described by Garnier et al. (2018).

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2.3. Optimization of culture conditions for antifungal activity enhancement

171 Bacterial (n=12) and fungal (n=1) fermentates exhibiting the highest antifungal activity were 172 selected for an optimization step of culture conditions. Depending on the considered 173 microorganism, 5 time/temperature combinations were tested: 20, 48 and 72 h at 30°C as well 174 as 48 h at 30°C followed by 24 h at either 12°C or 43°C for LAB; 48, 72 and 96 h at 30°C as 175 well as 96 h at 30°C followed by 24 h at either 12°C or 43°C for propionibacteria and 48, 96 176 and 168 h at 25°C as well as 96 h at 25°C followed by 48 h at either 12°C or 43°C for 177 filamentous fungi. Cell concentration at the end of incubation was determined on MRS and 178 YEL agar for LAB and propionibacteria, respectively. Antifungal activities of the resulting 179 fermentates were tested as described above against the four selected target fungal spoilers.

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- 181 2.4. In situ *antifungal activity*
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2.4.1. Antifungal activity by fermentate surface-spraying on semi-hard cheese

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184 Fermentates with promising antifungal activities were tested in situ by surface-spraying on 185 semi-hard cheeses (Table 2). Two replicate cheese trials were carried out at a pilot scale on 186 two separate weeks at the Dairy Platform of INRA (Rennes, France). Cow's milk was pasteurized at 72°C for 20 s, standardized to 30 g fat/kg milk and pumped into a cylindrical 187 188 jacketed, stainless steel cheese vat, with variable speed cutting and stirring (Frominox, Assat, 189 France). Milk was supplemented with 36 mg Ca per kg (20 mL per 100 kg milk of a 500 g/L 190 CaCl₂ solution). A commercial starter culture consisting of *Lactococcus lactis* subsp. *lactis* 191 and Lactococcus lactis subsp. cremoris (MA016, Elimeca, Thoissey, France) was

192 resuspended in UHT semi-skimmed milk, left to rehydrate for 1 h at 20°C and added to the cheese milk at 0.5 U/100 kg, i.e. ~10⁶ CFU/mL. After 2 h at 12°C followed by 30 min pre-193 ripening at 33°C (pH 6.57), 0.25 mL/kg of animal rennet (520 mg/L chymosin, Carlina 194 195 145/80, Dupont Danisco, Dangé, Saint Romain, France), diluted in deionized water, was 196 added. After coagulation for 29 min, curd was cut at the size of corn grains, washed with hot 197 water, cooked for 20 min at 35 °C and drained (pH 6.54). Curd was pre-pressed at 120 kPa for 198 30 min, moulded (700 g curd per mould) and pressed at 300 kPa for 30 min then 600 kPa for 199 90 min. The cheeses remained overnight in the mould, the temperature decreased from $\sim 25^{\circ}$ C 200 to $\sim 20^{\circ}$ C and the pH reached 5.07 at demoulding. Cheese composition at this step was 50.8 % 201 dry matter and 49 % fat in dry matter. Cheeses were salted by immersion in a sterile brine 202 containing 33 % (w/w) NaCl, 36 mg Ca per kg, pH 5 at 20 °C for 2 h. Cheeses were left to 203 dry for one night at room temperature (20-25°C). Then, in a laminar air flow cabinet, the 204 external surface (3 mm in thickness) was removed to limit surface contamination. Cheeses 205 were then smeared once with a mixture of the following commercial cultures: Debaryomyces 206 hansenii CHOOZIT DH, Kluyveromyces lactis CHOOZIT KL71, and Brevibacterium linens 207 CHOOZIT SR3 (Danisco, Dangé Saint-Romain, France). After 2 h drying at room 208 temperature in a laminar air flow cabinet, 3 ml of fermentates (bacterial lyophilized 209 fermentates being resuspended either in sterile distilled water or in filter-sterilized M. 210 *lanceolatus* fermentate), alone or in combination, were sprayed on the cheese surface. On day 211 2, after a 12h drying period at room temperature following the first spraying, cheese surface 212 was inoculated with 50 spores of either P. commune UBOCC-A-116003 or M. racemosus 213 UBOCC-A 116002. Fermentate spraying was then repeated on day 3 and 5. Cheeses were 214 ripened for 6 weeks in ripening chambers at 12°C and 96% relative humidity (one ripening 215 chamber per fungal target). The ripened semi-hard cheeses had a final dry matter of 60% and 216 pH 5.5. Fungal growth was visually evaluated every day during the 6 ripening weeks. A 217 negative control (cheese without surface fermentate spraying) and a positive control (cheese 218 surface treated with 1 mg/dm^2 natamycin on day 2) were also included.

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220 2.4.2. Antifungal activity by fermentate incorporation in sour cream

221 Various fermentates, alone or in combination, were also tested by incorporation in sour cream 222 (Table 2). To do so, cream (final fat 33%, dry matter 41%) was produced at a pilot scale from pasteurized milk (16s at 75°C) and fermented at 23°C for 22h using 2.5 U/100 kg (10⁶ 223 224 CFU/L) of commercial starter cultures MM100 (Elimeca, Thoissey, France). Sour cream (pH 225 4.5) was then packaged in individual 100g containers. Various fermentate concentrations 226 ranging from 0.1 to 5 % were tested according to the selected fermentate (Table 2). The 227 fermentates were added directly in each container, gently mixed and stored at 10°C overnight 228 before fungal target inoculation. A negative control (sour cream without fermentate) and a 229 positive control (sour cream containing 0.08 % potassium sorbate) were also included. For 230 challenge tests with filamentous fungi, 50 fungal spores of P. commune UBOCC-A-116003 or 231 M. racemosus UBOCC-A 116002 were inoculated on the sour cream surface followed by incubation at 10°C for 4 weeks. Fungal growth was visually evaluated every day. For 232 233 challenge test with Rhodotorula mucilaginosa UBOCC-A-216004, 2 CFU/g were inoculated 234 in the sour cream, followed by gentle mixing and incubation at 10°C. Yeast growth was 235 evaluated by surface-plating after 1, 2 and 3 weeks on YGC agar incubated at 25°C for 2 236 days.

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2.4.3. Durability tests

Durability tests were performed to evaluate fermentate antifungal activity in naturally contaminated environments. For semi-hard cheese, growth of spoilage fungi was evaluated on the surface of 3 different cheeses sprayed or not with selected fermentates and after ripening for 6 weeks at 12°C in a ripening chamber. For sour cream, 100 g of sour cream with or without fermentate, and a control containing 0.08 % potassium sorbate were spread out in 10 Petri dishes, exposed for 20 min to indoor air of the lunch room at INRA STLO and then sealed using parafilm. Fungal spoilage at the surface was visually evaluated after 2-weeks incubation at 10°C.

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2.4.4. Sensory analyses

249 To evaluate the potential impact of antifungal fermentates on the sensorial properties of 250 tested products, a sorting task was performed by a panel of 39 and 29 untrained judges for 251 cheese and sour cream, respectively. Sour cream, packed in 100 g containers, was kept at 4°C 252 for 2 weeks before sensory analysis and semi-hard cheese were tested after a 6 week ripening 253 period. Products were left for 30 min at room temperature before being served and a random 254 3-digit code was assigned to each sample. Panelists were asked to group together the samples 255 perceived as the most similar, taking into account the characteristics they considered as 256 important to differentiate the products (free sorting task). Once groups were made, panelists 257 were asked to associate specific descriptors to each group (verbalization task). To ensure the 258 panelist ability to group similar samples, a random sample was tested in duplicate. Sorting 259 task data were analyzed by the Factorial Approach for Sorting Task data (FAST) using 260 multiple correspondence analysis (MCA) via the FactoMineR and SensoMineR packages 261 implemented in the R environment, according to Cadoret et al. (2009). In particular, 262 confidence ellipses were drawn to also provide validation elements

To define the concentration of the selected fermentate at which no sensory impact could be perceived in sour cream, an acceptability test was performed with a panel of 26 untrained judges. Eight fermentate concentrations ranging from 0 to 0.7 % were tested. As for sensory tests, a random 3-digit code was assigned to each sample and products were left for 30 min at room temperature before being served. Panelists were asked to indicate whether each sourcream sample was acceptable or not for consumption.

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270 **3. Results**

271 3.1. Antifungal activity of fermentates after in vitro screening

In the first part of this work, the antifungal activity of fermentates obtained by individual culture of 698 LAB, propionibacteria or fungi in both LH and UF substrates (thus for a total of 1 396 fermentates) was evaluated *in vitro* against 4 fungal targets in a cheese-mimicking model. The number of fermentates exhibiting antifungal activity largely varied according to the substrate used for fermentation, the tested taxon, and the fungal target (Supplementary table S2).

278 LH fermentates were overall notably more inhibitory than UF fermentates with LAB 279 being the most represented microbial group among active strains. Regarding LH fermentates, 280 81 (18.9 %), 13 (3 %), and 1 (0.2 %) LAB fermentates out of the 430 tested ones, were active 281 against P. commune, M. racemosus, and Y. lipolytica, respectively, while no propionibacteria 282 out of the 70 tested showed any activity. For fungal fermentates, 2 (1 %) and 6 (3 %) out of 283 the 198 tested ones were active against *M. racemosus* and *G. geotrichum*, respectively. In 284 contrast, for UF fermentates, only fermentates from 8 LAB (1.8% of tested LAB), 1 285 propionibacteria (1.4 % of tested propionibacteria) and 4 fungi (2 % of tested fungi) showed 286 an antifungal activity, and this against only one target.

Among the 27 tested bacterial species, *Lactobacillus buchneri* (n=34), *Lactobacillus plantarum* (n=115) and *Lactobacillus brevis* (n=8) represented the most active species with 44.1, 40.8 and 37.5 % of active LH fermentates against at least one target. Noteworthy, 9 LAB LH fermentates showed an antifungal activity against both *M. racemosus* and *P. commune*, while a *L. buchneri* L162 fermentate inhibited the latter 2 fungi and *Y. lipolytica*

as well. In contrast, in the tested conditions, only a few strains of *Lactobacillus reuteri* (n=42)
and *Propionibacterium jensenii* (n=20) showed antifungal activity, with 1 (2.4 %) and 1 (5 %)
active strains for LH and UF fermentates, respectively. In addition, *Leuconostoc mesenteroides* (n=71), *Propionibacterium acidipropionici* (n=17) and *Propionibacterium thoenii* (n=14) strains did not show any antifungal activity whatever the substrate used.

297 As mentioned above, only a few fungal fermentates showed antifungal activities and there 298 was no particular over-represented species whatever the substrate used. Indeed, for LH 299 fermentates, one isolate of Aureobasidium pullulans and M. lanceolatus, and Exophiala sp., 300 Rhodotorula minuta, Rhodotorula mucilaginosa, Torulaspora delbrueckii and Trichoderma 301 viride, led to fermentates active against M. racemosus and G. geotrichum, respectively. For 302 UF fermentates, one isolate of Actinomucor elegans, Verticillium dahliae and Candida tenuis, 303 and Trichoderma harzianum produced fermentates active against P. commune and Y. 304 *lipolytica*, respectively.

305 Concerning the susceptibility of the fungal targets, *P. commune*, followed by *M.* 306 *racemosus* were the most frequently inhibited fungi, while *G. geotrichum* and *Y. lipolytica* 307 were only inhibited by a very small number of LAB or propionibacteria fermentates. 308 Interestingly, for fungal fermentates, *M. racemosus* and *G. geotrichum* were the most 309 sensitive targets with LH as a growth substrate whereas it was *P. commune* and *Y. lipolytica* 310 with UF substrate.

After *in vitro* screening, 11 strains exhibiting the most promising antifungal activity,
namely 9 *Lactobacillus* strains (*L. buchneri* L151, L162, L164 and L233, *L. harbinensis*L172, *L. paracasei* L117 and L194, *L. plantarum* L244 and *L. rhamnosus* CIRM-BIA1952), *P. jensenii* CIRM-BIA1774 and *M. lanceolatus* UBOCC-A-109193 were selected for further
investigation.

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318 The 11 strains previously selected were inoculated and cultivated in LH and/or UF substrate 319 with 5 different time/temperature combinations, and the antifungal activity of the obtained 320 fermentates were tested against the 4 selected fungal targets in the cheese model. Increases in 321 antifungal activity against at least one fungal target were observed for all strains when culture 322 conditions were modified (Table 1). Activity improvement was more pronounced against 323 P. commune, followed by R. mucilaginosa and M. racemosus, whereas there was little 324 improvement against Y. lipolytica. The increase in time before visible growth, which varied 325 between 1 to 4 days as compared to the control depending on the tested strain, was generally 326 higher in conditions with prolonged incubation time with the exception of *L. paracasei* L117 327 fermentate for which incubation for 20 h at 30°C yielded the best antifungal activity. 328 Increases in antifungal activity were also generally related to the final cell concentration at the 329 end of the incubation even though this was not systematically observed. Finally, a final 330 incubation step at suboptimal temperatures did not improve the antifungal activity except for 331 L buchneri L233, M. lanceolatus UBOCC-A-109193 and P. jensenii CIRM-BIA1774 332 fermentates for which a significant increase in antifungal activity against P. commune and 333 *M. racemosus*, and *M. racemosus* and *R. mucilaginosa*, respectively, was observed (Table 1). 334 After this step, 3 fermentates were selected for *in situ* applications at a pilot scale, namely 335 those of L. rhamnosus CIRM-BIA1952 (cultivated in LH for 48 h at 30°C) which was able to

delay *P. commune* and *R. mucilaginosa* growth, *P. jensenii* CIRM-BIA1774 (cultivated in UF

337 for 96 h at 30°C followed by 24 h at 12°C), which was able to delay the growth of the 4 tested

fungal targets, and *M. lanceolatus* UBOCC-A-109193 (cultivated in LH for 96 h at 30°C

followed by 48 h at 12°C) which was able to delay *M. racemosus* and *P. commune* growth.

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3.3. Antifungal activity and organoleptic impact of fermentates after surface-spraying on semi-hard cheeses

343 The 3 fermentates selected after the optimization step were used, alone or in combination 344 (either L. rhamnosus or P. jensenii fermentate associated with M. lanceolatus fermentate), for 345 surface treatment of semi-hard cheeses manufactured at a pilot-scale. The observed times to 346 visible growth of *M. racemosus* and *P. commune* after the challenge test are presented in 347 Table 2. Surface-spraying of each of the tested fermentates led to an increase in time to visible 348 growth of both fungal targets as compared to the negative control (untreated cheese), but their 349 efficiency varied. For example, the L. rhamnosus / M. lanceolatus and P. jensenii / 350 *M. lanceolatus* combinations increased the time to visible growth of *M. racemosus* by 3 and 351 15 days, respectively. The same combinations increased the time to visible growth of 352 P. commune by 2 and 7 days, respectively. The most efficient fermentate was that of 353 P. jensenii CIRM-BIA1774, which inhibited M. racemosus and P. commune for up to 21 and 354 14 days, respectively. No visible growth of the fungal targets was observed after natamycin 355 application during the time of the experiment (33 days). Interestingly, we did not observe any 356 effect of the fermentates on the smear development, while that of the cheese treated with 357 natamycin was considerably reduced (data not shown).

The strong antifungal activity of the fermentates after surface-treatment of semi-hard cheeses was further confirmed by the durability test results. As shown in Figure 1, control cheeses (untreated) were almost completely covered with mold after 6 week incubation at 10°C in a naturally contaminated ripening chamber, whereas the fermentate treated cheeses appeared with a much smaller surface covered by mold, with the exception of 1 out of 3 replicate treated with *P. jensenii* fermentate.

The results of the multiple correspondence analysis made from cheese sensory data are presented in Figure 2. The total variance explained by the first two dimensions was 62 %, with Dim 1 and Dim 2 representing 34 and 28 % of variance, respectively. The panelists could differentiate all cheeses except the cheese treated with *P. jensenii* fermentate which could not be differentiated from other samples including the control cheese.

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370 3.4. Antifungal activity and organoleptic impact of fermentates after incorporation in sour
 371 cream

372 Tested concentrations were initially 2 and 5 % (w/w) of each fermentate and a mixture of both 373 fermentates at 2.5 % (w/w) each. Once again, the P. jensenii CIRM-BIA1774 fermentate, 374 alone or in combination with L. rhamnosus CIRM-BIA1952 fermentate, showed a high antifungal activity (similar to that of potassium sorbate) against M. racemosus and 375 376 P. commune. It delayed their growth up to 30 days (total duration of the experiment) as 377 compared to the negative control (Table 2). All fermentates containing *P. jensenii* totally 378 inhibited R. mucilaginosa growth (counts <1 CFU/g) during storage, while that of 379 L. rhamnosus did not show any antifungal activity (Figure 3).

Durability tests confirmed the trends observed with the challenge tests as none of the samples containing *P. jensenii* CIRM-BIA1774 fermentate showed any visible fungal contamination (Figure 4). In contrast, samples containing *L. rhamnosus* CIRM-BIA1952 fermentates at 2 and 5 % were only slightly less contaminated than control samples with 90 and 80 % of samples showing visible fungal contamination, respectively.

In the multiple correspondence analysis, the total variance explained by the two first dimensions was 61 %, with Dim 1 and Dim 2 representing 37 and 24 % of variance, respectively (Figure 5). The overlapping of the confidence ellipses of the control sample and sour cream with 2 % *L. rhamnosus* fermentate showed that these samples were perceived as similar by the panelists. Samples containing *P. jensenii* fermentates were grouped together and clearly separated from the control on Dim 1, while sour creams containing 5 % *L*. 391 *rhamnosus* CIRM-BIA1952 fermentate were separated from the control on Dim 2, indicating 392 that panelists could perceive significant sensory differences between these samples. Sour 393 creams with *P. jensenii* CIRM-BIA1774 fermentate were described as very unpleasant, 394 strong, and acid, showing a high negative impact of this fermentate on the product flavor 395 while sour creams with 5 % *L. rhamnosus* CIRM-BIA1952 fermentate were described as dry, 396 pasty or burnt showing an impact on texture and flavour.

397 Based on these results, using an acceptability test, we investigated the concentration of 398 P. jensenii fermentate at which no organoleptic impact could be perceived in sour cream 399 (Supplementary Figure 1). Acceptability decreased with increasing fermentate concentrations. 400 Among the 26 panelists, 21 (80.8 %) accepted the control sour cream while only 6 (23.1 %) 401 accepted the product with 0.7 % P. jensenii fermentate. The 21 panelists who did not reject 402 the sour cream also accepted the sour cream containing 0.1 % P. jensenii fermentate, while 10 403 panelists (47.6%) accepted that containing 0.4% P. jensenii fermentate. These 2 404 concentrations were selected for further challenge and durability tests.

For challenge tests in sour creams containing 0.1 and 0.4 % *P. jensenii* fermentate, fungal growth delays were much shorter than those observed at 2 and 5 %. They corresponded to 1 and 3 days, and 3 and 4 days for *M. racemosus* and *P. commune*, respectively (Table 2). On the contrary, no antifungal activity was observed against *R. mucilaginosa* (Figure 3). Concerning durability tests, all control sour creams were contaminated, as well as sour creams with 0.1% fermentate (Figure 6). Interestingly, none of the samples with 0.4% fermentate showed any visible fungal contamination (Figure 6).

412

413 **4. Discussion**

414

415 In order to develop dairy antifungal biopreservation ingredients, fermentation of LH and UF was performed by large array of microorganisms. LH and FH were chosen for their 416 417 availability since they are current products of the dairy industry, food-grade status and 418 compatibility with dairy food applications. The *in vitro* screening step of corresponding 419 fermentates revealed that several fermentates exhibited a significant antifungal activity. 420 Interestingly, the proportion of active fermentates and their antifungal activity varied 421 according to the dairy substrate. Better expression of antifungal activity was observed for LH 422 substrate (qualitatively and/or quantitatively) as compared to UF substrate. The antifungal 423 activity was not correlated to the ability of the selected microorganisms to grow in each of these sustrates. Indeed, for a dedicated strain, an assessment of the final biomass on the tested 424 425 27 bacterial and 18 fungal strains showed that similar populations were generally reached in both substrates after fermentation $(10^7 - 10^9 \text{ CFU/ml} \text{ for bacteria and } 10^7 \text{ CFU/ml} \text{ for yeasts})$, 426 427 except for filamentous fungi which generally showed ~5 times more final biomass in LH than 428 in UF (data not shown). Therefore, for most bacteria and yeasts, it is likely that the 429 differential expression in the production of antifungal activity was related to the substrate 430 composition, while, for filamentous fungi, both biomass level and/or substrate composition 431 could be involved. The individual components which are responsible remain unclear. It can be 432 underlined however that LH but not UF substrate contains milk fat and is richer in proteins, 433 both qualitatively (presence of caseins) and quantitatively (374 g/kg total nitrogen and 190 434 g/kg nonprotein nitrogen in LH versus 5.7 g/kg total nitrogen and 5.5 g/kg non-protein 435 nitrogen in UF). Fat and proteins are precursors of antifungal compounds such as fatty acids, 436 peptides, organic acids, and volatile compounds (Hidalgo et al., 2015, Théolier et al., 2013). 437 Similar observations were made in a recent study showing that production of lactic, acetic, 438 succinic, propionic, formic, and butyric acids by LAB significantly varied according to the 439 growth medium used (Özcelik et al., 2016). Concerning the proportion of antifungal strains

observed in the present study, 17 % of LH and 1 % UF fermentates showed a significant 440 441 antifungal activity against at least one fungal target. These proportions are much lower than 442 those found in previous studies. Inglin et al. (2015) found that ~30 % of 504 tested lactobacilli 443 isolates, cultivated in MRS agar, inhibited at least one of the 4 tested fungal targets. More 444 recently, Le Lay et al. (2016) reported that ~32 % of the 270 tested LAB and 50 tested 445 propionibacteria inhibited 5 fungal targets after growth in wheat flour hydrolysate agar. The 446 lower number of active strains reported in the present study can be explained by the fact that 447 fermentates instead of bacterial colonies (Inglin et al., 2015) or bacteria embedded in agar 448 medium (Le Lay et al. 2016) were used, probably resulting in a more stringent selection of 449 active isolates. In addition, the substrates used for screening were also different, for example, 450 Inglin et al. (2015) utilized MRS medium containing acetate, which is known to potentialize 451 antifungal activity (Stiles et al., 2002) while in Le Lay et al. (2016) work, pH of wheat flour hydrolysate medium after LAB growth was ~3.5 (1.5 unit lower to miniaturized cheeses's 452 453 pH), which enhances the antifungal activity of organic acids. In this study the testing matrix 454 (cheese-mimicking model) was more closely related to an actual food product.

Our results confirmed the strain-dependent character of antifungal activities in terms of percent of active strains as well as inhibited targets. Indeed, within each screened species, strains significantly differed in their antifungal activity as reported elsewhere (Le Lay et al., 2016, Cortés-Zavaletta et al. 2014, Thierry et al., 2015, Russo et al., 2017, Leyva-Salas et al., 2017). For example, for *L. buchneri* (n=34), one of the most antifungal species, around 40% of strains were active, while in *L. reuteri* (n=42), only 2 % of the strains were active.

Antifungal fermentate-producing isolates mostly belonged to *Lactobacillus* species such as *L. brevis* (Axel et al. 2016), *L. buchneri* (Kharazian et al. 2017), *L.plantarum* (Cheong et al.
2014), *L. harbinensis* (Belguesmia et al. 2014), and *L. rhamnosus* (Fernandez et al. 2017).

464 This can be explained by the fact that *Lactobacillus* spp. produce a large range of antifungal

molecules such as organic acids (Brosnan et al., 2012), hydrogen peroxide, ethanol, cyclic 465 466 dipeptides, proteinaceous compounds and fatty acids (Luz et al., 2017; Crowley et al., 2013; 467 Lavermicocca et al., 2000). The additive or even synergistic action of these molecules, despite 468 being produced at weak concentrations, could increase the antifungal activity. Interestingly, 469 one Propionibacterium and 7 fungal strains also produced antifungal fermentates. 470 Propionibacteria produce propionic acid, as well as hydrogen peroxide, azelaic, phenyllactic 471 and hydroxyphenyllactic acids (Schwenninger et al., 2004, Fernandez et al., 2017; Le Lay et 472 al., 2016) and fungi are known to produce antifungal compounds acting in synergy such as 473 volatile compounds, killer proteins, antifungal peptides and lytic enzymes (Leyva-Salas et al., 474 2017, Nùñez et al., 2015, Izgu et al., 2011, Coelho et al., 2009; Weiler and Schmitt, 2003; 475 Wheatley, 2002; Schmitt et al., 1996) (see also the review by Delgado et al., 2016). To our 476 best knowledge, this is the first time that A. pullulans, C. tenuis and M. lanceolatus are 477 reported for their antifungal activities. In contrast, Trichoderma spp. such as T. harzianum 478 and T. viride are commonly used as biocontrol agents and are known to produce both non-479 volatile and volatile compounds like pyrones, butenolides, azaphylones, anthraquinones, 480 trichothecenes, terpenoids and steroids, as well as non-ribosomal peptides, such as 481 siderophores and peptaibols (Mukherjee et al., 2012). In future studies, it would be interesting 482 to understand which antifungal molecules are involved in the antifungal activity of the most 483 active fermentates and to investigate their mode of action against different fungal targets. 484 In order to enhance antifungal activities an optimization step of culture conditions was 485 included. In general, the longer the incubation time and the higher the cell concentration was,

486 the more the antifungal activity was significant. In addition to incubation time, cold or hot 487 thermal shock increased the antifungal activity of several strains. The reasons for this are 488 unclear but suboptimal temperatures could trigger or increase the production of antifungal 489 compounds as seen for example for bacteriocin production in LAB (Leal-Sánchez et al.490 2002).

491 When selecting strains for food applications, microbial safety and regulatory 492 considerations such as Generally Recognized as Safe (GRAS) or Qualified Presumption of 493 Safety (QPS) must be considered (Bourdichon et al., 2012). Thus, the safety of the LAB and 494 propionibacteria strains selected for *in situ* tests were verified, i.e. biogenic amines production 495 and antibiotic resistance pattern. L. brevis L128 and L. buchneri L162 and L233, although 496 showing interesting antifungal properties were not kept further for *in situ* step applications 497 since they were shown to possess biogenic amines associated genes (hdc and agdi for L162 498 and L233, agdi and tyrdc for L128) and to produce histamine, putrescine and tyramine in 499 vitro (Coton et al. 2018). On the contrary, P. jensenii CIRM-BIA1774 and L. rhamnosus 500 CIRM-BIA1952, selected for the *in situ* tests were both considered as safe for dairy food 501 applications. Concerning Mucor, while some species are mycosis agents, others are 502 technological agents in food products and known not to produce mycotoxins (Morin-Sardin et 503 al. 2017). More specifically, M. lanceolatus has a long history of safe use in food as it is utilized as a ripening culture in Tomme and Saint-Nectaire cheeses (Desmasures, 2014, 504 505 Hermet et al., 2014). In situ tests including challenge and durability tests are a crucial step in 506 the process of developing an antifungal ingredient since in vitro screening can lead to an 507 overestimation of the antifungal activity as discussed above (see also Levva-Salas et al. 2017) 508 For challenge tests, surface-spraying of P. jensenii CIRM-BIA1774 fermentate on the cheese 509 surface was efficient for delaying fungal growth without any impact on cheese sensory 510 characterisitics. Noteworthy, to our best knowledge, it is the first time that the use of a 511 fermentate is shown to efficiently replace natamycin. Further work would be necessary in an 512 industrial context to verify its antifungal activity in other cheese types as well as to assess 513 whether it can be produced on a large scale with an acceptable cost. In sour cream, P. jensenii 514 fermentate at 2 and 5 % (w/w) harbored a very high antifungal activity against the 3 tested 515 targets and prevented fungal contaminations of natural origin for up to one month. However, 516 at these high concentrations, its negative impact on sensory attributes, yielding very acid sour 517 cream with propionic odor and flavor mainly linked to high concentration of propionic acid, is 518 a major limit for its application in this product category. Nevertheless, it is interesting to 519 underline that it prevented natural contaminations at 0.4 % (w/w) with a limited impact on 520 consumer acceptance. Further work is still necessary to evaluate whether it could be used or 521 not in this product type. These results also indicate that, before performing time-consuming 522 sensorial analysis, a preliminary study to determine the maximum concentration to be used 523 without any impact on sensorial properites could have been useful. Volatile profiles could 524 also be determined on antifungal fermentates prior to sensory analyses and on products 525 subjected to sensorial analysis to develop rapid screening and quality control approaches, 526 using for example head space mass spectrometry (Makhoul et al., 2016). The use of 527 L. rhamnosus CIRM-BIA1952 fermentate, incorporated in sour cream or sprayed on semi-528 hard cheeses, had no impact on product sensorial properties but yielded a limited antifungal 529 activity. However, when considering durability tests results the L. rhamnosus fermentate were 530 much more promising. It is worth mentioning that, for *in situ* challenge tests, 50 fungal spores 531 were inoculated, which is a worst-case scenario in comparison to natural contamination and 532 that tested products were incubated at 10°C, which is also favorable to fungal growth.

533 Concerning *M. lanceolatus*, to our knowledge, this is the first time that the antifungal activity
534 of a *M. lanceolatus* fermentate is demonstrated.

535

536 **5. Conclusion**

537 To our best knowledge, this study is the first one which describes the development of dairy538 based antifungal ingredients for a large number of strains and species, from *in vitro* screening

to pilot scale applications, including the evaluation of their impact on product organoleptic properties. The obtained results provided 3 promising antifungal dairy which may be used in dairy foods as part of the hurdle technology and to replace or at least limit the use of chemical preservatives such as natamycin or potasium sorbate. From a fundamental point of view, the identification of the molecules harboring antifungal activities and their mechanism of action should be explored.

545

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| fermentate. | | | | Incr | ease in time to vi | isible fungal growth (| (in days) |
|---------------------------|---------------------|------------------------|---------------------------------------|-----------------|--------------------|------------------------|---------------|
| Fermentation substrate | Strain | Culture conditions* | Cell concentration (log 10 CFU/ml) | M. racemosus | P. commune | R. mucilaginosa | Y. lipolytica |
| Low heat milk | L. buchneri L151 | 1-a | 5.10^{7} | 0 | 0 | 0 | 0 |
| | | 2-a | 6.10^{7} | 0 | 0 | + | 0 |
| | | 3-a | 2.10^{8} | 0 | + | +2 | 0 |
| | | 4-a | 6.10^{7} | 0 | 0 | +1 | 0 |
| | | 5-a | 7.10^{7} | 0 | 0 | +2 | 0 |
| | L. buchneri L162 | 1-a | 1.10^{7} | 0 | 0 | +1 | 0 |
| | | 2-a | 4.10^{8} | 0 | +1 | +1 | 0 |
| | | 3-a | 1.10^{8} | 0 | $\dot{\omega}^+$ | +1 | 0 |
| | | 4-a | 8.10^{8} | 0 | \dot{c}^+ | + | 0 |
| | | 5-a | 1.10^{9} | 0 | +4 | +2 | 0 |
| | L. buchneri L164 | 1-a | 6.10^{8} | 0 | +1 | +1 | 0 |
| | | 2-a | 3.10^{8} | 0 | $^+1$ | +1 | 0 |
| | | 3-a | 2.10^{8} | 0 | 0 | +1 | 0 |
| | | 4-a | 4.10^{8} | 0 | +1 | +1 | 0 |
| | | 5-a | 2.10^{8} | 0 | 0 | +1 | 0 |
| | L. buchneri L233 | 1-a | 6.10^{7} | 0 | +1 | 0 | 0 |
| | | 2-a | 7.10^{6} | 0 | $^{+1}$ | 0 | 0 |
| | | 3-a | 5.10^{8} | $^+1$ | +3 | +1 | 0 |
| | | 4-a | 2.10^{8} | 0 | +2 | +1 | 0 |
| | | 5-a | 4.10^{8} | $^{+1}$ | +4 | +2 | 0 |
| | L. harbinensis L172 | 1-a | 2.10^{8} | $^{+1}$ | 0 | +1 | 0 |
| | | 2-a | 7.10^{7} | 0 | 0 | 0 | 0 |
| | | 3-a | 8.10^{7} | 0 | 0 | +2 | 0 |
| | | 4-a | 2.10^{8} | 0 | 0 | +1 | 0 |
| | | 5-a | 1.10^{8} | $^{+1}$ | 0 | +1 | 0 |
| | L. paracasei L117 | 1-3 | 2.10^{9} | + | +4 | 5+ | 0 |

merrol without against M. racemosus UBOCC-A-116002, P. commune UBOCC-A-116003, R. mucilaginosa UBOCC-A-216004 and Y. lipolytica UBOCC-A-216006. The antifunced portivity is expressed as increased in time to visible arouth of the funced terms (days) as command to a control without Table 1. Effect of culture conditions on the antifungal activity of fermentates from selected lactic acid bacteria, propionibacteria and fungi

| | | 2-a | 2.10^{9} | 0 | +1 | +2 | 0 |
|-------------------------------|--|--|---|-------------------------------------|-------------------------------------|---|------------------|
| | | 3-a | 1.10^{8} | 0 | +1 | +1 | 0 |
| | | 4-a | 1.10^{9} | 0 | +1 | +2 | 0 |
| | | 5-a | 2.10^{9} | 0 | 0 | +1 | 0 |
| | L. paracasei L194 | 1-a | 3.10^{9} | 0 | +2 | +2 | 0 |
| | | 2-a | 2.10^{9} | 0 | +1 | +1 | 0 |
| | | 3-a | 3.10^{8} | 0 | +1 | +1 | 0 |
| | | 4-a | 2.10^{9} | 0 | +1 | +1 | 0 |
| | | 5-a | 2.10^{9} | 0 | +1 | +1 | 0 |
| | L. plantarum L244 | 1-a | 7.10^{8} | 0 | +1 | +1 | 0 |
| | | 2-a | 5.10^{8} | 0 | +1 | +1 | 0 |
| | | 3-a | 2.10^{8} | 0 | 0 | +1 | 0 |
| | | 4-a | 6.10^{8} | 0 | +1 | +1 | 0 |
| | | 5-a | 1.10^{8} | 0 | +1 | +1 | 0 |
| | L. rhannosus CIRM-BIA1952 | 1-a | 2.10^{9} | 0 | +2 | +2 | 0 |
| | | 2-a | 1.10^{9} | 0 | +3 | +2 | 0 |
| | | 3-a | 1.10^{9} | 0 | +3 | +1 | 0 |
| | | 4-a | 1.10^{9} | 0 | +1 | +1 | 0 |
| | | 5-a | 2.10^{9} | 0 | $^{+1}$ | +2 | 0 |
| | M. lanceolatus UBOCC-A-109193 | 1-b | thu | $^{+1}$ | 0 | nd | 0 |
| | | 2-b | nd | +2 | +1 | nd | 0 |
| | | 3-b | nd | $^{+1}$ | +1 | nd | 0 |
| | | 4-b | nd | +4 | +1 | nd | 0 |
| | | 5-b | nd | 0 | +1 | nd | 0 |
| UF milk | L. buchneri L162 | 1-a | 1.10^{7} | 0 | 0 | +1 | 0 |
| permeate | | 2-a | 4.10^{8} | 0 | 0 | +1 | 0 |
| | | 3-a | 1.10^{8} | 0 | 0 | 0 | 0 |
| | | 4-a | 8.10^{8} | 0 | 0 | 0 | 0 |
| | | 5-a | 1.10^{9} | 0 | 0 | 0 | 0 |
| | P. jensenii CIRM-BIA1774 | 1-c | 1.10^{7} | $^{+1}$ | +1 | +1 | 0 |
| | | 2-c | 2.10^{8} | +2 | +1 | +1 | 0 |
| | | 3-c | 6.10^{8} | +2 | +1 | +2 | 0 |
| | | 4-c | 8.10^{8} | +2 | +1 | +2 | +1 |
| | | 5-c | 8.10^{8} | +2 | $^{+1}$ | +1 | 0 |
| *Culture col conditions fi | nditions for lactobacilli: 1-a, 24h/30°C; 2-a, 48 or <i>M. lanceolatus</i> UBOCC-A-109193: 1-b, 48l | h/30°C; 3-a, 72h/30 v/25°C; 2-b, 96h/25 | 0°C; 4-a, 48h/30°C 5°C; 3-b, 168h/25°C | ollowed by 24h/ 3; 4-b, 96h/25°C | (12°C; 5-a, 48h/3 followed by 48 | 30°C followed by 2/ 3h/12°C; 5-b, 96h/25 | th/43°C. Culture |

48h/43°C. Culture conditions for *P. jensenii* CIRM-BIA1774: 1-c, 48h/30°C; 2-c, 72h/30°C; 3-c, 96h/30°C; 4-c, 96h/30°C followed by 24h/12°C; 5-c, 96h/30°C followed by 24h/43°C; nd, not determined. 749 750

Table 2. Antifungal activity of selected fermentates against *M. racemosus* UBOCC-A-116002 and *P. commune* UBOCC-A-116003 in semi-hard cheese and sour cream.

| | | | Time before visible | fungal growth (in days) | |
|----------------|--|--|---------------------|-------------------------|--|
| Product | Fermentate (fermentation substrate) | Concentration | M. racemosus | P. commune | |
| əs | None (negative control) | | 5 | L | |
| зәә ц ә | P. jensenii CIRM-BIA1774 (UF) and M. lanceolatus UBOCC-A-109193 (LH) | $0.63 \text{ g/dm}^2 \text{ and}$ 5 ml/dm^2 | 20 | 14 | |
| ցւզ | P. jensenii CIRM-BIA1774 (UF) | 0.63 g/dm ² | 26 | 21 | |
| sd-im9 | L. rhamnosus CIRM-BIA1952 (LH) and M. lanceolatus UBOCC-A-109193 (LH) | 1.26 g/dm ² and 5 ml/dm ² | 8 | 6 | |
| S | Natamycin (positive control) | 1 mg/dm ² | >33 | >33 | |
| | None (negative control) | | Δ | 8 | |
| | L. rhannosus CIRM-BIA1952 (LH) | 5% | 7 | 8 | |
| | L. rhannosus CIRM-BIA1952 (LH) | 2% | 7 | 8 | |
| uu | P. jensenii CIRM-BIA1774 (UF) | 5% | 30 | 30 | |
| s913 | P. jensenii CIRM-BIA1774 (UF) | 2% | 30 | 19 | |
| nr c | P. jensenii CIRM-BIA1774 (UF) | 0.4% | 10 | 12 | |
| os | P. jensenii CIRM-BIA1774 (UF) | 0.1% | 8 | 11 | |
| | L. rhamnosus CIRM-BIA1952 (LH) and P. jensenii CIRM-BIA1774 (UF) | 2.5% each | >30 | >30 | |
| | Potassium sorbate (positive control) | 0.08% | >30 | >30 | |



Figure 1. Photographs showing semi-hard cheese surfaces treated or not (negative control,
Nc) by surface-spraying using *P. jensenii* CIRM-BIA1774 (Pj), *P. jensenii* CIRM-BIA1774
associated with *M. lanceolatus* UBOCC-A-109193 (Pj +Ml) or *L. rhamnosus* CIRM-BIA1952
associated with *M. lanceolatus* UBOCC-A-109193 (Lr + Ml), after 6 weeks of ripening at
10°C in a naturally contaminated environment. Numbers 1 to 3 are replicates.



Dim 1 (34.06%)

763 764 Figure 2. Impact of cheese surface treatment with antifungal fermentates on sensorial 765 properties of semi-hard cheese determined using a sorting approach: representation of the cheeses and their respective 95 % confidence ellipses in the plane defined by Dim 1 and Dim 766 2 of a multiple correspondence analysis. A1 and A2, control cheeses (without fermentate); B, 767 cheese surface-treated with a combination of 4.8 mg/dm² L. rhamnosus CIRM-BIA1952 and 768 10 ml/dm² M. lanceolatus UBOCC-A-109193 fermentates; C, cheese surface-treated with a 769 combination of 2.4 mg/dm² P. jensenii CIRM-BIA1774 and 10 ml/dm² M. lanceolatus 770 UBOCC-A-109193 fermentates and D, cheese surface-treated with 2.4 mg/dm² P. jensenii 771 772 CIRM-BIA1774 fermentate.





Figure 3. Growth of *R. mucilaginosa* UBOCC-A-216004 in sour cream after incorporation or
not of *P. jensenii* CIRM-BIA1774 and/or *L. rhamnosus* CIRM-BIA1952 fermentates. Nc,
negative control (sour cream without fermentate); Pc, positive control (sour cream with 0.08
% potassium sorbate); Pj-2, *P. jensenii* fermentate at 2 % w/w; Pj-5, *P. jensenii* fermentate at
5 % w/w, Lr-2, *L. rhamnosus* fermen-tate at 2 % w/w; L-5, *L. rhamnosus* fermentate at 5 %
w/w; Pj-2.5+Lr-2.5, mixture of *P. jensenii* and *L. rhamnosus* fermentates at 2.5 % w/w each.



782 Figure 4. Photographs showing sour cream surfaces in which fermentates of P. jensenii 783 CIRM-BIA1774 (2 % and 5%), L. rhamnosus CIRM-BIA1952 (2 and 5 %) and mixture of P. jensenii and L. rhamnosus at 2.5 % each were incorporated. Negative controls 784 785 corresponded to sour cream samples without fermentate. Samples were stored for 2 weeks at 786 4°C, naturally contaminated in an indoor environment (20 min) and stored again for 2 weeks 787 at 10°C. Nc, Negative control; Pj-2, P. jensenii fermentate at 2% w/w; Pj-5, P. jensenii 788 fermentate at 5 % w/w, Lr-2, L. rhamnosus fermentate at 2% w/w; Lr-5, L. rhamnosus 789 fermentate at 5 % w/w; Pj-2.5 + Lr-2.5, mixture of *P. jensenii* and *L. rhamnosus* fermentates 790 at 2.5 % w/w each. Numbers 1 to 10 are replicates. Score at the bottom of the figure 791 correspond to the number of contaminated cream / total number of cream.

792



793 794 Figure 5. Impact of the incorporation of antifungal fermentates on sensorial traits of sour 795 cream determined using a sorting approach: representation of the sour creams and their 796 respective 95 % confidence ellipses in the plane defined by Dim 1 and Dim 2 of a multiple 797 correspondence analysis. A, control sour cream (without fermentate); B1 and B2, sour cream 798 with 2 % L. rhamnosus CIRM-BIA1952 fermentate, C, sour cream with 5 % L. rhamnosus 799 CIRM-BIA1952 fermentate; D, sour cream with 2 % P. jensenii CIRM-BIA1774 fermentate, 800 E, sour cream with 5 % of P. jensenii CIRM-BIA1774 fermentate; F, sour cream with 2.5 % 801 L. rhamnosus CIRM-BIA1952 and 2.5 % of P. jensenii CIRM-BIA1774 fermentates.



Figure 6. Photographs showing sour cream surfaces in which P. jensenii CIRM-BIA1774 fermentates were incorporated at 0.1 (Pj-0.1) and 0.4 (Pj-0.4) %. Negative control (Nc) correspond to sour cream samples without fermentate and positive control (Pc) to sour cream samples containing 0.08 % potassium sorbate. Samples were stored for 2 weeks at 4°C, naturally contaminated in an indoor environment and stored again for 2 weeks at 10°C. Numbers 1 to 10 are replicates. Scores correspond to number of contaminated sour cream/ total number of sour cream. 803 804 805 806

807

802

808 Supplementary data

809 Supplementary Table S1. List of tested microorganisms

| Species | Strain | Species | Strain |
|---------------------------|--------------|-------------------------------|--------|
| Lactobacillus acidophilus | CIRM-BIA443 | Lactobacillus casei/paracasei | L101 |
| Lactobacillus acidophilus | CIRM-BIA445 | Lactobacillus casei/paracasei | L102 |
| Lactobacillus acidophilus | CIRM-BIA448 | Lactobacillus casei/paracasei | L103 |
| Lactobacillus acidophilus | CIRM-BIA1674 | Lactobacillus casei/paracasei | L106 |
| Lactobacillus amylovorus | CIRM-BIA669 | Lactobacillus casei/paracasei | L107 |
| Lactobacillus brevis | L128 | Lactobacillus casei/paracasei | L108 |
| Lactobacillus brevis | L30 | Lactobacillus casei/paracasei | L109 |
| Lactobacillus brevis | L31 | Lactobacillus casei/paracasei | L110 |
| Lactobacillus brevis | CIRM-BIA608 | Lactobacillus casei/paracasei | L112 |
| Lactobacillus brevis | CIRM-BIA932 | Lactobacillus casei/paracasei | L117 |
| Lactobacillus brevis | CIRM-BIA933 | Lactobacillus casei/paracasei | L118 |
| Lactobacillus brevis | CIRM-BIA1523 | Lactobacillus casei/paracasei | L119 |
| Lactobacillus brevis | CIRM-BIA1531 | Lactobacillus casei/paracasei | L120 |
| Lactobacillus buchneri | L111 | Lactobacillus casei/paracasei | L121 |
| Lactobacillus buchneri | L137 | Lactobacillus casei/paracasei | L122 |
| Lactobacillus buchneri | L147 | Lactobacillus casei/paracasei | L124 |
| Lactobacillus buchneri | L149 | Lactobacillus casei/paracasei | L127 |
| Lactobacillus buchneri | L151 | Lactobacillus casei/paracasei | L129 |
| Lactobacillus buchneri | L162 | Lactobacillus casei/paracasei | L13 |
| Lactobacillus buchneri | L164 | Lactobacillus casei/paracasei | L130 |
| Lactobacillus buchneri | L169 | Lactobacillus casei/paracasei | L136 |
| Lactobacillus buchneri | L170 | Lactobacillus casei/paracasei | L138 |
| Lactobacillus buchneri | L192 | Lactobacillus casei/paracasei | L139 |
| Lactobacillus buchneri | L203 | Lactobacillus casei/paracasei | L140 |
| Lactobacillus buchneri | L212 | Lactobacillus casei/paracasei | L142 |
| Lactobacillus buchneri | L217 | Lactobacillus casei/paracasei | L143 |
| Lactobacillus buchneri | L233 | Lactobacillus casei/paracasei | L144 |
| Lactobacillus buchneri | L239 | Lactobacillus casei/paracasei | L148 |
| Lactobacillus buchneri | L243 | Lactobacillus casei/paracasei | L150 |
| Lactobacillus buchneri | L245 | Lactobacillus casei/paracasei | L157 |
| Lactobacillus buchneri | L25 | Lactobacillus casei/paracasei | L158 |
| Lactobacillus buchneri | L265 | Lactobacillus casei/paracasei | L159 |
| Lactobacillus buchneri | L271 | Lactobacillus casei/paracasei | L16 |
| Lactobacillus buchneri | L273 | Lactobacillus casei/paracasei | L161 |
| Lactobacillus buchneri | L277 | Lactobacillus casei/paracasei | L179 |
| Lactobacillus buchneri | L278 | Lactobacillus casei/paracasei | L184 |
| Lactobacillus buchneri | L290 | Lactobacillus casei/paracasei | L186 |
| Lactobacillus buchneri | L56 | Lactobacillus casei/paracasei | L188 |
| Lactobacillus buchneri | L84 | Lactobacillus casei/paracasei | L19 |
| Lactobacillus buchneri | L90 | Lactobacillus casei/paracasei | L194 |
| Lactobacillus buchneri | L99 | Lactobacillus casei/paracasei | L196 |
| Lactobacillus buchneri | CIRM-BIA659 | Lactobacillus casei/paracasei | L199 |
| Lactobacillus buchneri | CIRM-BIA1324 | Lactobacillus casei/paracasei | L208 |
| Lactobacillus buchneri | CIRM-BIA1514 | Lactobacillus casei/paracasei | L209 |
| Lactobacillus buchneri | CIRM-BIA1515 | Lactobacillus casei/paracasei | L211 |
| Lactobacillus buchneri | CIRM-BIA1516 | Lactobacillus casei/paracasei | L215 |

| Lactobacillus buchneri | CIRM-BIA1525 | Lactobacillus casei/paracasei | L220 |
|-------------------------------|--------------|-------------------------------------|---------------|
| Lactobacillus casei/paracasei | L1 | Lactobacillus casei/paracasei | L222 |
| Lactobacillus casei/paracasei | L100 | Lactobacillus casei/paracasei | L224 |
| Lactobacillus casei/paracasei | L225 | Lactobacillus casei/paracasei | L69 |
| Lactobacillus casei/paracasei | L226 | Lactobacillus casei/paracasei | L7 |
| Lactobacillus casei/paracasei | L227 | Lactobacillus casei/paracasei | L72 |
| Lactobacillus casei/paracasei | L228 | Lactobacillus casei/paracasei | L73 |
| Lactobacillus casei/paracasei | L229 | Lactobacillus casei/paracasei | L74 |
| Lactobacillus casei/paracasei | L23 | Lactobacillus casei/paracasei | L75 |
| Lactobacillus casei/paracasei | L230 | Lactobacillus casei/paracasei | L82 |
| Lactobacillus casei/paracasei | L231 | Lactobacillus casei/paracasei | L85 |
| Lactobacillus casei/paracasei | L232 | Lactobacillus casei/paracasei | L86 |
| Lactobacillus casei/paracasei | L234 | Lactobacillus casei/paracasei | L93 |
| Lactobacillus casei/paracasei | L237 | Lactobacillus casei/paracasei | L95 |
| Lactobacillus casei/paracasei | L24 | Lactobacillus delbrueckii | L160 |
| Lactobacillus casei/paracasei | L240 | Lactobacillus delbrueckii | L218 |
| Lactobacillus casei/paracasei | L242 | Lactobacillus farciminis | CIRM-BIA1634 |
| Lactobacillus casei/paracasei | L247 | Lactobacillus fermentum | CIRM-BIA42 |
| Lactobacillus casei/paracasei | L249 | Lactobacillus fermentum | CIRM-BIA666 |
| Lactobacillus casei/paracasei | L250 | Lactobacillus gasseri | CIRM-BIA665 |
| Lactobacillus casei/paracasei | L252 | Lactobacillus gasseri | CIRM-BIA1437 |
| Lactobacillus casei/paracasei | L253 | Lactobacillus gasseri | CIRM-BIA1438 |
| Lactobacillus casei/paracasei | L257 | Lactobacillus gasseri | CIRM-BIA1447 |
| Lactobacillus casei/paracasei | L261 | Lactobacillus harbinensis | L172 |
| Lactobacillus casei/paracasei | L263 | Lactobacillus pentosus | CIRM-BIA660 |
| Lactobacillus casei/paracasei | L264 | Lactobacillus pentosus | CIRM-BIA853 |
| Lactobacillus casei/paracasei | L266 | Lactobacillus pentosus | CIRM-BIA854 |
| Lactobacillus casei/paracasei | L267 | Lactobacillus pentosus | CIRM-BIA1490 |
| Lactobacillus casei/paracasei | L269 | Lactobacillus periolens/harbinensis | L191 |
| Lactobacillus casei/paracasei | L27 | Lactobacillus perolens/harbinensis | L244 |
| Lactobacillus casei/paracasei | L270 | Lactobacillus perolens/harbinensis | L283 |
| Lactobacillus casei/paracasei | L274 | Lactobacillus plantarum | L10 |
| Lactobacillus casei/paracasei | L276 | Lactobacillus plantarum | L11 |
| Lactobacillus casei/paracasei | L279 | Lactobacillus plantarum | L12 |
| Lactobacillus casei/paracasei | L281 | Lactobacillus plantarum | L123 |
| Lactobacillus casei/paracasei | L284 | Lactobacillus plantarum | L125 |
| Lactobacillus casei/paracasei | L287 | Lactobacillus plantarum | L14 |
| Lactobacillus casei/paracasei | L288 | Lactobacillus plantarum | L145 |
| Lactobacillus casei/paracasei | L289 | Lactobacillus plantarum | L146 |
| Lactobacillus casei/paracasei | L3 | Lactobacillus plantarum | L15 |
| Lactobacillus casei/paracasei | L32 | Lactobacillus plantarum | L152 |
| Lactobacillus casei/paracasei | L32 L37 | Lactobacillus plantarum | L155 |
| Lactobacillus casei/paracasei | L 38 | Lactobacillus plantarum | L155 |
| Lactobacillus casei/paracasei | L30 I 40 | Lactobacillus plantarum | L163 |
| Lactobacillus casei/paracasei | L40 I 47 | Lactobacillus plantarum | L167 |
| Lactobacillus casei/paracasei | I 51 | | I 17 |
| Lactobacillus casei/paracasei | L 57 | | Γ171 |
| Lactobacillus casei/paracasei | L52 I 57 | | I 173 |
| Lactobacillus casei/paracasei | L 58 | Lactobacillus plantarum | L173 I 174 |
| Lactobacillus casei/paracasei | I 59 | Lactobacillus plantarum | L174 I 175 |
| Lactobacillus casei/paracasei | L57 I 61 | Lactobacillus plantarum | L175 I 176 |
| Lactodacuius casei/paracasei | LUI | Lactobacillus plantarum | L170 |

| Lactobacillus casei/paracasei | L62 | Lactobacillus plantarum | L18 |
|-------------------------------|------|-------------------------|--------------|
| Lactobacillus casei/paracasei | L68 | Lactobacillus plantarum | L180 |
| Lactobacillus plantarum | L181 | Lactobacillus plantarum | L43 |
| Lactobacillus plantarum | L182 | Lactobacillus plantarum | L45 |
| Lactobacillus plantarum | L183 | Lactobacillus plantarum | L46 |
| Lactobacillus plantarum | L193 | Lactobacillus plantarum | L48 |
| Lactobacillus plantarum | L195 | Lactobacillus plantarum | L49 |
| Lactobacillus plantarum | L198 | Lactobacillus plantarum | L5 |
| Lactobacillus plantarum | L20 | Lactobacillus plantarum | L50 |
| Lactobacillus plantarum | L200 | Lactobacillus plantarum | L53 |
| Lactobacillus plantarum | L201 | Lactobacillus plantarum | L53 |
| Lactobacillus plantarum | L202 | Lactobacillus plantarum | L54 |
| Lactobacillus plantarum | L204 | Lactobacillus plantarum | L6 |
| Lactobacillus plantarum | L205 | Lactobacillus plantarum | L6 |
| Lactobacillus plantarum | L206 | Lactobacillus plantarum | L63 |
| Lactobacillus plantarum | L207 | Lactobacillus plantarum | L64 |
| Lactobacillus plantarum | L21 | Lactobacillus plantarum | L65 |
| Lactobacillus plantarum | L210 | Lactobacillus plantarum | L65 |
| Lactobacillus plantarum | L216 | Lactobacillus plantarum | L66 |
| Lactobacillus plantarum | L219 | Lactobacillus plantarum | L70 |
| Lactobacillus plantarum | L22 | Lactobacillus plantarum | L71 |
| Lactobacillus plantarum | L221 | Lactobacillus plantarum | L76 |
| Lactobacillus plantarum | L241 | Lactobacillus plantarum | L77 |
| Lactobacillus plantarum | L246 | Lactobacillus plantarum | L8 |
| Lactobacillus plantarum | L248 | Lactobacillus plantarum | L87 |
| Lactobacillus plantarum | L251 | Lactobacillus plantarum | L88 |
| Lactobacillus plantarum | L254 | Lactobacillus plantarum | L88 |
| Lactobacillus plantarum | L255 | Lactobacillus plantarum | L89 |
| Lactobacillus plantarum | L256 | Lactobacillus plantarum | L9 |
| Lactobacillus plantarum | L258 | Lactobacillus plantarum | L96 |
| Lactobacillus plantarum | L259 | Lactobacillus plantarum | L98 |
| Lactobacillus plantarum | L26 | Lactobacillus plantarum | CIRM-BIA466 |
| Lactobacillus plantarum | L260 | Lactobacillus plantarum | CIRM-BIA652 |
| Lactobacillus plantarum | L268 | Lactobacillus plantarum | CIRM-BIA845 |
| Lactobacillus plantarum | L272 | Lactobacillus plantarum | CIRM-BIA850 |
| Lactobacillus plantarum | L28 | Lactobacillus plantarum | CIRM-BIA855 |
| Lactobacillus plantarum | L282 | Lactobacillus plantarum | CIRM-BIA1105 |
| Lactobacillus plantarum | L285 | Lactobacillus plantarum | CIRM-BIA1106 |
| Lactobacillus plantarum | L286 | Lactobacillus plantarum | CIRM-BIA1108 |
| Lactobacillus plantarum | L29 | Lactobacillus plantarum | CIRM-BIA1109 |
| Lactobacillus plantarum | L291 | Lactobacillus plantarum | CIRM-BIA1110 |
| Lactobacillus plantarum | L292 | Lactobacillus plantarum | CIRM-BIA1111 |
| Lactobacillus plantarum | L293 | Lactobacillus plantarum | CIRM-BIA1419 |
| Lactobacillus plantarum | L294 | Lactobacillus plantarum | CIRM-BIA1420 |
| Lactobacillus plantarum | L298 | Lactobacillus plantarum | CIRM-BIA1520 |
| Lactobacillus plantarum | L299 | Lactobacillus reuteri | L154 |
| Lactobacillus plantarum | L35 | Lactobacillus reuteri | L165 |
| Lactobacillus plantarum | L36 | Lactobacillus reuteri | L177 |
| Lactobacillus plantarum | L39 | Lactobacillus reuteri | L178 |
| Lactobacillus plantarum | L4 | Lactobacillus reuteri | L185 |
| Lactobacillus plantarum | L41 | Lactobacillus reuteri | L187 |

| Lactobacillus plantarum | L41 | Lactobacillus reuteri | L189 |
|--------------------------------|---------------|----------------------------|--------------|
| Lactobacillus reuteri | L190 | Lactococcus lactis | L168 |
| Lactobacillus reuteri | L197 | Lactococcus lactis | L2 |
| Lactobacillus reuteri | L213 | Leuconostoc citreum | CIRM-BIA742 |
| Lactobacillus reuteri | L214 | Leuconostoc citreum | CIRM-BIA852 |
| Lactobacillus reuteri | L223 | Leuconostoc citreum | CIRM-BIA1453 |
| Lactobacillus reuteri | L235 | Leuconostoc citreum | CIRM-BIA1454 |
| Lactobacillus reuteri | L236 | Leuconostoc citreum | CIRM-BIA1455 |
| Lactobacillus reuteri | L238 | Leuconostoc citreum | CIRM-BIA1456 |
| Lactobacillus reuteri | L262 | Leuconostoc citreum | CIRM-BIA1457 |
| Lactobacillus reuteri | L275 | Leuconostoc mesenteroïdes | L104 |
| Lactobacillus reuteri | L280 | Leuconostoc mesenteroïdes | L113 |
| Lactobacillus reuteri | L295 | Leuconostoc mesenteroïdes | L114 |
| Lactobacillus reuteri | L296 | Leuconostoc mesenteroïdes | L115 |
| Lactobacillus reuteri | L297 | Leuconostoc mesenteroïdes | L116 |
| Lactobacillus reuteri | L33 | Leuconostoc mesenteroïdes | L126 |
| Lactobacillus reuteri | L44 | Leuconostoc mesenteroïdes | L131 |
| Lactobacillus reuteri | L55 | Leuconostoc mesenteroïdes | L132 |
| Lactobacillus reuteri | L60 | Leuconostoc mesenteroïdes | L133 |
| Lactobacillus reuteri | L67 | Leuconostoc mesenteroïdes | L134 |
| Lactobacillus reuteri | L78 | Leuconostoc mesenteroïdes | L135 |
| Lactobacillus reuteri | L79 | Leuconostoc mesenteroïdes | L141 |
| Lactobacillus reuteri | L80 | Leuconostoc mesenteroïdes | CIRM-BIA694 |
| Lactobacillus reuteri | L81 | Leuconostoc mesenteroïdes | CIRM-BIA740 |
| Lactobacillus reuteri | 1.83 | Leuconostoc mesenteroïdes | CIRM-BIA847 |
| Lactobacillus reuteri | L.91 | Leuconostoc mesenteroïdes | CIRM-BIA1146 |
| Lactobacillus reuteri | 1.92 | Leuconostoc mesenteroïdes | CIRM-BIA1147 |
| Lactobacillus reuteri | L94 | Leuconostoc mesenteroïdes | CIRM-BIA1153 |
| Lactobacillus reuteri | L97 | Leuconostoc mesenteroïdes | CIRM-BIA1154 |
| Lactobacillus reuteri | CIRM-BIA522 | Leuconostoc mesenteroïdes | CIRM-BIA1155 |
| | CIRM-BIA696 | Leuconostoc mesenteroïdes | CIRM-BIA1156 |
| | CIRM-BIA911 | Leuconostoc mesenteroïdes | CIRM-BIA1159 |
| | CIRM-BIA912 | Leuconostoc mesenteroïdes | CIRM-BIA1176 |
| | CIRM-BIA929 | Leuconostoc mesenteroïdes | CIRM-BIA1185 |
| | CIRM-BIA1439 | Leuconostoc mesenteroïdes | CIRM-BIA1187 |
| | CIRM-BIA1534 | Leuconosto e mesenteroides | CIRM-BIA1191 |
| | CIRM-BIA607 | Leuconosto e mesenteroides | CIRM-BIA1192 |
| Lactobacillus rhamnosus | CIRM-BIA909 | Leuconosto e mesenteroides | CIRM-BIA1193 |
| Lactobacillus rhamnosus | CIRM-BIA910 | Leuconosto e mesenteroides | CIRM-BIA1194 |
| Lactobacillus rhamnosus | CIRM-BIA913 | Leuconosto e mesenteroides | CIRM-BIA1228 |
| | CIRM-BIA930 | | CIRM-BIA1220 |
| | CIRM-BIA1107 | | CIRM-BIA1220 |
| Lactobaculus rhamnosus | CIRM-BIA1117 | | CIRM-BIA1230 |
| Lactobaculus rhamnosus | CIRM-BIA1112 | | CIRM-BIA1232 |
| Lactobaculus rhamnosus | CIRM-BIA1/136 | | CIRM-BIA1232 |
| Lactobacillus rnamnosus | CIRM_RIA/67 | Leuconostoc mesenterolaes | CIRM_RIA1223 |
| Lactobaculus sakel | CIDM BIA807 | Leuconostoc mesenteroides | CIDM DIA1234 |
| Lactobaculus sakel | CIRM RIA1550 | Leuconostoc mesenteroides | CIDM DIA1233 |
| Lactobacillus sakei | CIRM DIA1339 | Leuconostoc mesenteroïdes | |
| Lactobacillus sanfranciscensis | CIDM DIA1557 | Leuconostoc mesenteroïdes | CIDM DIA123/ |
| Lactobacillus zeae | CIDM DIA525 | Leuconostoc mesenteroïdes | CIRM DIA1238 |
| Lactobacillus zeae | UKWI-DIA323 | Leuconostoc mesenteroïdes | UKWI-BIA1239 |

Leuconostoc mesenteroïdes Pediococcus pentosaceus Pediococcus pentosaceus Propionibacterium acidipropionici Propionibacterium freudenreichii Propionibactrium thoenii

CIRM-BIA1242 CIRM-BIA1244 CIRM-BIA1245 CIRM-BIA1246 CIRM-BIA1249 CIRM-BIA1250 CIRM-BIA1251 CIRM-BIA1252 CIRM-BIA1259 CIRM-BIA1260 CIRM-BIA1261 CIRM-BIA1262 CIRM-BIA1264 CIRM-BIA1265 CIRM-BIA1266 CIRM-BIA1267 CIRM-BIA1268 CIRM-BIA1269 CIRM-BIA1270 CIRM-BIA1271 CIRM-BIA1272 CIRM-BIA1302 CIRM-BIA1304 CIRM-BIA1305 CIRM-BIA1314 CIRM-BIA1315 CIRM-BIA1317 CIRM-BIA1318 CIRM-BIA1320 CIRM-BIA1326 L42 CIRM-BIA1701 CIRM-BIA64 CIRM-BIA609 CIRM-BIA928 CIRM-BIA1384 CIRM-BIA1424 CIRM-BIA1425 CIRM-BIA1427 CIRM-BIA1535 CIRM-BIA1652 CIRM-BIA1656 CIRM-BIA1664 CIRM-BIA1762 CIRM-BIA1763 CIRM-BIA1764 CIRM-BIA1765 CIRM-BIA1769 CIRM-BIA1770 CIRM-BIA10 CIRM-BIA1791

Propionibacterium freudenreichii Propionibacterium jensenii Propionibactrium thoenii Gliocladium deliquescens

CIRM-BIA12 CIRM-BIA41 CIRM-BIA118 CIRM-BIA1324 CIRM-BIA141 CIRM-BIA508 CIRM-BIA509 CIRM-BIA510 CIRM-BIA512 CIRM-BIA514 CIRM-BIA515 CIRM-BIA527 CIRM-BIA683 CIRM-BIA686 CIRM-BIA687 CIRM-BIA688 CIRM-BIA690 CIRM-BIA692 CIRM-BIA39 CIRM-BIA455 CIRM-BIA1347 CIRM-BIA1431 CIRM-BIA1432 CIRM-BIA1494 CIRM-BIA1505 CIRM-BIA1507 CIRM-BIA1658 CIRM-BIA1660 CIRM-BIA1661 CIRM-BIA1774 CIRM-BIA1775 CIRM-BIA1777 CIRM-BIA1778 CIRM-BIA1779 CIRM-BIA1781 CIRM-BIA1782 CIRM-BIA1785 CIRM-BIA1794 CIRM-BIA40 CIRM-BIA1329 CIRM-BIA1423 CIRM-BIA1429 CIRM-BIA1433 CIRM-BIA1435 CIRM-BIA1500 CIRM-BIA1663 CIRM-BIA1786 CIRM-BIA1788 CIRM-BIA1789 CIRM-BIA1790 UBOCC-A-101174

Propionibactrium thoenii Actinomucor elegans Actinomucor elegans Actinomucor elegans Aureobasidium pullulans Candida catenulata Candida davisiane Candida haemulonis Candida methapsilosis Candida parapsilosis Candida parapsilosis Candida parapsilosis Candida pseudoglaebosa Candida sorbophila Candida tenuis Candida zeylanoides Candida zeylanoides Chaetomium dolichotrichum Chaetomium funicola Chaetomium globosum Cleistothelebolus nipigonensis Cryptococcoccus curvatus Cryptococcoccus curvatus Cryptococcocus albidus Cryptococcocus diffluens Cryptococcus magnus Cryptococcus uzbekistanensis Cystofilobasidium infirmominiatum Debaryomyces hansenii Debaryomyces hansenii Exophiala castellanii Exophiala dermatitidis Exophiala sp. Exophiala xenobiotica Phoma betae Phoma glomerata

CIRM-BIA1793 UBOCC-A-101333 UBOCC-A-102005 UBOCC-A-106035 UBOCC-A-101091 UBOCC-A-101092 UBOCC-A-108047 UBOCC-A-108048 UBOCC-A-108056 UBOCC-A-108057 UBOCC-A-111234 L1lev3 UBOCC-A-214090 UBOCC-A-214059 L1lev6 UBOCC-A-214083 L1lev5 UBOCC-A-214060 UBOCC-A-214089 UBOCC-A-214111 UBOCC-A-214064 L2lev6 UBOCC-A-214114 UBOCC-A-101010 UBOCC-A-101011 UBOCC-A-101012 UBOCC-A-214079 UBOCC-A-214084 UBOCC-A-214094 UBOCC-A-214081 UBOCC-A-214070 UBOCC-A-214092 UBOCC-A-214131 UBOCC-A-214077 L1lev4 UBOCC-A-214074 UBOCC-A-214056 UBOCC-A-214076 UBOCC-A-101133 UBOCC-A-108008 UBOCC-A-108012 UBOCC-A-108017 UBOCC-A-108021 UBOCC-A-108022 UBOCC-A-108025 UBOCC-A-108026 UBOCC-A-108035 UBOCC-A-108059 UBOCC-A-214088 UBOCC-A-111053 UBOCC-A-101141

Gliocladium roseum Gliocladium sp. Gliocladium virens Gliomastix murorum Gliomastix murorum Lodderomyces elongisporus Meyerozyma guilliermondii Meyerozyma guilliermondii Meyerozyma guilliermondii Meyerozyma guilliermondii Mucor bruneogriseus Mucor fragilis Mucor fragilis Mucor fragilis Mucor fuscus Mucor fuscus Mucor fuscus Mucor fuscus Mucor fuscus Mucor griseocyanus Mucor hiemalis Mucor hiemalis Mucor hiemalis Mucor hiemalis Mucor hiemalis Mucor hiemalis Mucor lanceolatus Mucor mucedo Mucor mucedo Mucor plumbeus Mucor plumbeus Mucor plumbeus Mucor plumbeus Mucor plumbeus Mucor plumbeus Mucor racemosus Mucor racemosus Mucor racemosus Mucor racemosus Mucor racemosus Mucor racemosus Mucor sp. Mucor sp. Schizosaccharomyces pombe Schizosaccharomyces pombe

UBOCC-A-101175 UBOCC-A-102025 UBOCC-A-101176 UBOCC-A-101178 UBOCC-A-101179 UBOCC-A-214062 L1lev7 L2lev17 UBOCC-A-214105 UBOCC-A-208004 UBOCC-A-109204 UBOCC-A-109205 UBOCC-A-109206 JBOCC-A-109207 UBOCC-A-109208 UBOCC-A-109209 UBOCC-A-109210 UBOCC-A-109196 UBOCC-A-109199 UBOCC-A-113030 UBOCC-A-101355 UBOCC-A-109194 UBOCC-A-109195 UBOCC-A-109202 UBOCC-A-110148 UBOCC-A-102010 UBOCC-A-101359 UBOCC-A-101360 UBOCC-A-102003 UBOCC-A-109197 UBOCC-A-111119 UBOCC-A-112185 UBOCC-A-109193 UBOCC-A-101361 UBOCC-A-101362 UBOCC-A-109203 UBOCC-A-111125 UBOCC-A-111126 UBOCC-A-111128 UBOCC-A-111129 UBOCC-A-111132 UBOCC-A-101366 UBOCC-A-102017 UBOCC-A-109186 UBOCC-A-109211 UBOCC-A-109212 UBOCC-A-109213 UBOCC-A-110121 UBOCC-A-112143 UBOCC-A-201052 UBOCC-A-201053

Phoma sp. Phoma sp. Phoma valerianellae Phoma valerianellae Phoma valerianellae Pichia anomala Pichia anomala Pichia anomala Pichia anomala Pichia anomala Pichia burtonii Pichia fermentans Pichia fermentans Pichia membranifaciens Pichia norvegensis Pseudozyma sp. Rhizomucor pusillus Rhizomucor pusillus Rhodotorula laryngis Rhodotorula minuta Rhodotorula mucilaginosa Rhodotorula mucilaginosa Rhodotorula mucilaginosa Rhodotorula mucilaginosa Rhodotorula slooffiae Rhodotorula slooffiae Rhodotorula vanillica Saccharomyces bailii Saccharomyces bailii Saccharomyces bailii Saccharomyces carlsbergensis Saccharomyces carlsbergensis Saccharomyces carlsbergensis Saccharomyces cerevisiae Saccharomyces kluyveri Saccharomyces ludwigii Saccharomyces rouxii Saccharomyces uvarum Saccharomyces uvarum Saccharomyces uvarum Saccharomyces uvarum Saccharomyces uvarum

UBOCC-A-102026 UBOCC-A-109091 UBOCC-A-101242 UBOCC-A-101243 UBOCC-A-101244 UBOCC-A-201001 UBOCC-A-205011 UBOCC-A-212007 UBOCC-A-212008 UBOCC-A-212011 UBOCC-A-212009 UBOCC-A-201032 UBOCC-A-201033 UBOCC-A-214071 UBOCC-A-201034 UBOCC-A-214138 UBOCC-A-101365 UBOCC-A-111202 UBOCC-A-214057 UBOCC-A-214082 UBOCC-A-214069 UBOCC-A-214075 UBOCC-A-214091 UBOCC-A-214102 UBOCC-A-214103 UBOCC-A-214073 UBOCC-A-214104 UBOCC-A-201004 UBOCC-A-201035 UBOCC-A-205008 UBOCC-A-201036 UBOCC-A-201038 UBOCC-A-205034 UBOCC-A-214061 UBOCC-A-201006 UBOCC-A-201039 UBOCC-A-201040 UBOCC-A-201041 UBOCC-A-205009 UBOCC-A-201042 UBOCC-A-201045 UBOCC-A-205010 UBOCC-A-201046 UBOCC-A-201047 UBOCC-A-201048 UBOCC-A-201049 UBOCC-A-201050 UBOCC-A-201051

Sporobolomyces roseus Sporobolomyces roseus Torulaspora delbrueckii Torulaspora delbrueckii Torulaspora delbrueckii Torulaspora delbrueckii Trichoderma harzianum Trichoderma harzianum Trichoderma harzianum Trichoderma harzianum Trichoderma koningii Trichoderma viride Trichoderma viride Trichoderma viride Trichoderma viride Trichosporon asahii Trichosporon coremiiforme Trichosporon dulcitum Trichosporon montevideense Verticillium albo-atrum Verticillium albo-atrum Verticillium albo-atrum Verticillium albo-atrum Verticillium albo-atrum Verticillium dahliae Verticillium intertextum Verticillium lecanii Verticillium lecanii Verticillium lecanii Verticillium nigrescens Verticillium nonalfalfae Verticillium tricorpus Wickerhamomyces anomalus Zygosaccharomyces bailii Zygosaccharomyces mellis Zygosaccharomyces rouxii Zygosaccharomyces rouxii Zygosaccharomyces sp.

UBOCC-A-214093 UBOCC-A-214078 L2lev7 L2lev3 L1lev2 UBOCC-A-214128 UBOCC-A-101288 UBOCC-A-101289 UBOCC-A-111253 UBOCC-A-111254 UBOCC-A-101290 UBOCC-A-101292 UBOCC-A-111251 UBOCC-A-111252 UBOCC-A-112152 UBOCC-A-214080 UBOCC-A-214058 UBOCC-A-214085 UBOCC-A-214068 UBOCC-A-101303 UBOCC-A-101304 UBOCC-A-101305 UBOCC-A-101306 UBOCC-A-101307 UBOCC-A-101308 UBOCC-A-101309 UBOCC-A-101310 UBOCC-A-101311 UBOCC-A-101312 UBOCC-A-101313 UBOCC-A-101314 UBOCC-A-101315 UBOCC-A-101316 UBOCC-A-101317 UBOCC-A-101318 UBOCC-A-101319 UBOCC-A-101320 UBOCC-A-108019 UBOCC-A-108023 UBOCC-A-101322 UBOCC-A-112135 UBOCC-A-101323 UBOCC-A-214106 UBOCC-A-201062 UBOCC-A-201063 UBOCC-A-201064 UBOCC-A-209001 UBOCC-A-202008 **Supplementary Table S2**. Antifungal activity of fermentates from lactic acid bacteria, propionibacteria and fungi grown in low heat milk (LH) and ultrafiltration milk permeate (UF) against *M. racemosus* UBOCC-A-116002, *P. commune* UBOCC-A-116003, *G. geotrichum* UBOCC-A-216001 and *Y. lipolytica* UBOCC-A-216006 after *in vitro* screening in miniaturized cheeses.

| | | Num | ber of | f activ | ve* fe | rmentates | Nur | nber o | of activ | ve fer | mentates |
|------------------------------------|--------------------------|--------------|------------|---------------|---------------|-----------------------------------|--------------|------------|---------------|---------------|-----------------------------------|
| | | | ob | tained | l on L | Н | | ob | tained | on U | F |
| Taxon | Number of isolates | M. racemosus | P. commune | G. geotrichum | Y. lipolytica | More than one target (%) | M. racemosus | P. commune | G. geotrichum | Y. lipolytica | More than one target (%) |
| Lactobacillus acidophilus | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus amylovorus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus brevis | 8 | 1 | 3 | 0 | 0 | 1 (12.5) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus buchneri | 34 | 3 | 15 | 0 | 1 | 3 (8.8) | 0 | 3 | 0 | 0 | 0 (0) |
| Lactobacillus casei/paracasei | 112 | 4 | 12 | 0 | 0 | 2 (1.8) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus delbruecki | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus farciminis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus fermentum | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus gasseri | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus harbinensis | 1 | 1 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus pentosus | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus perolens/harbinensis | 3 | 1 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus plantarum | 115 | 3 | 47 | 0 | 0 | 3 (2.6) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus reuteri | 42 | 0 | 1 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus rhamnosus | 9 | 0 | 3 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus sakei | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 | 0 (0) |
| Lactobacillus sanfranciscensis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lactobacillus zeae | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 | 0 (0) |
| Lactococcus lactis | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 2 | 0 (0) |
| Leuconostoc citreum | 7 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 1 | 0 | 0 | 0 (0) |
| Leuconostoc mesenteroïdes | 71 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pediococcus pentosaceus | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Propionibacterium acidopropionici | 17 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Propionibacterium freudenreichii | 19 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Propionibacterium jensenii | 20 | 0 | 0 | 0 | 0 | 0 (0) | 1 | 0 | 0 | 0 | 0 (0) |
| Propionibacterium thoenii | 14 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Actinomucor elegans | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 1 | 0 | 0 | 0 (0) |
| Aureobasidium pullulans | 7 | 1 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida catenulata | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida davisiana | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida haemulonis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida methapsilosis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida parapsilosis | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida pseudoglaebosa | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida sorbophila | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Candida tenuis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 | 0 (0) |
| Candida zeylanoides | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |

| Chaetomium dolichotrichum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
|-----------------------------------|----|---|---|---|---|-------|---|---|---|---|-------|
| Chaetomium funicola | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Chaetomium globosum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cleistothelebolus nipigonensis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cryptococcus albidus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cryptococcus curvatus | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cryptococcus diffluens | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cryptococcus magnus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cryptococcus uzbekistanensis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Cystofilobasidium infirmominiatum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Debaryomyces hansenii | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Exophiala castellanii | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Exophiala dermatidis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Exophiala xenobiotica | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Exophiala sp. | 10 | 0 | 0 | 2 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Gliocladium deliquescens | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Gliocladium roseum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Gliocladium virens | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Gliocladium sp. | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Gliomastix murorum | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Lodderomyces elongisporus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Meyerozyma guilliermondii | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor brunneogriseus | 7 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor fragilis | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor fuscus | 5 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor griseocyanus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor hiemalis | 6 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor lanceolatus | 1 | 1 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor mucedo | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor plumbeus | 6 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor racemosus | 6 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Mucor sp. | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Phoma betae | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Phoma glomerata | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Phoma valerianellae | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Phoma sp. | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pichia anomala | 5 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pichia burtonii | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pichia fermentans | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pichia membranifaciens | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pichia norvegensis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Pseudozyma sp. | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhizomucor pusillus | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhodotorula laryngis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhodotorula minuta | 1 | 0 | 0 | 1 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhodotorula mucilaginosa | 4 | 0 | 0 | 1 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhodotorula slooffiae | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Rhodotorula vanillica | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Saccharomyces carlsbergensis | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |

| Total | | 698 | 15 | 81 | 6 | 1 | 9 (1.3) | 1 | 6 | 0 | 6 | 0 (0) |
|-----------------------|-----------|-----|----|----|---|---|---------|---|---|---|---|-------|
| Total fungi | | 198 | 2 | 0 | 6 | 0 | 0 (0) | 0 | 2 | 0 | 2 | 0 (0) |
| Total bacteria | | 500 | 13 | 81 | 0 | 1 | 9 (1.8) | 1 | 4 | 0 | 4 | 0 (0) |
| Zygosaccharomyce | s sp. | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Zygosaccharomyce | s rouxii | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Zygosaccharomyce | s mellis | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Zygosaccharomyce | s bailii | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Wickerhamomyces | anomalus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticiullium tricor | pus | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticillium nonalfa | ılfae | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticillium nigreso | ens | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticillium lecanii | | 3 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticillium intertex | ctum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Verticillium dahliae | | 11 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 1 | 0 | 0 | 0 (0) |
| Verticillium albo-ai | rum | 5 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichosporon monte | evideense | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichosporon dulci | tum | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichosporon coren | niiforme | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichosporon asahi | i | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichoderma viride | | 4 | 0 | 0 | 1 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichoderma koning | gii | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Trichoderma harzia | num | 4 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 1 | 0 (0) |
| Torulaspora delbru | eckii | 4 | 0 | 0 | 1 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Sporobomyces rose | us | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Schizosaccharomyc | es pombe | 2 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Saccharomyces uva | rum | 5 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Saccharomyces lud | wigii | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Saccharomyces klu | vveri | 1 | 0 | 0 | 0 | 0 | 0 (0) | 0 | 0 | 0 | 0 | 0 (0) |
| Sacona onlyces con | | | | | | | | | | | | |

* Delayed growth or total inhibition of the fungal target in comparison with the negative control.

Supplementary figure 1. Acceptability test for sour cream inoculated with increased concentrations of *P. jensenii* CIRM-BIA1774 fermentate ranging from 0.1 to 0.7 %. Results corresponding to the 21 of 26 panelists accepting the the control (sour cream without fermentate addition).

