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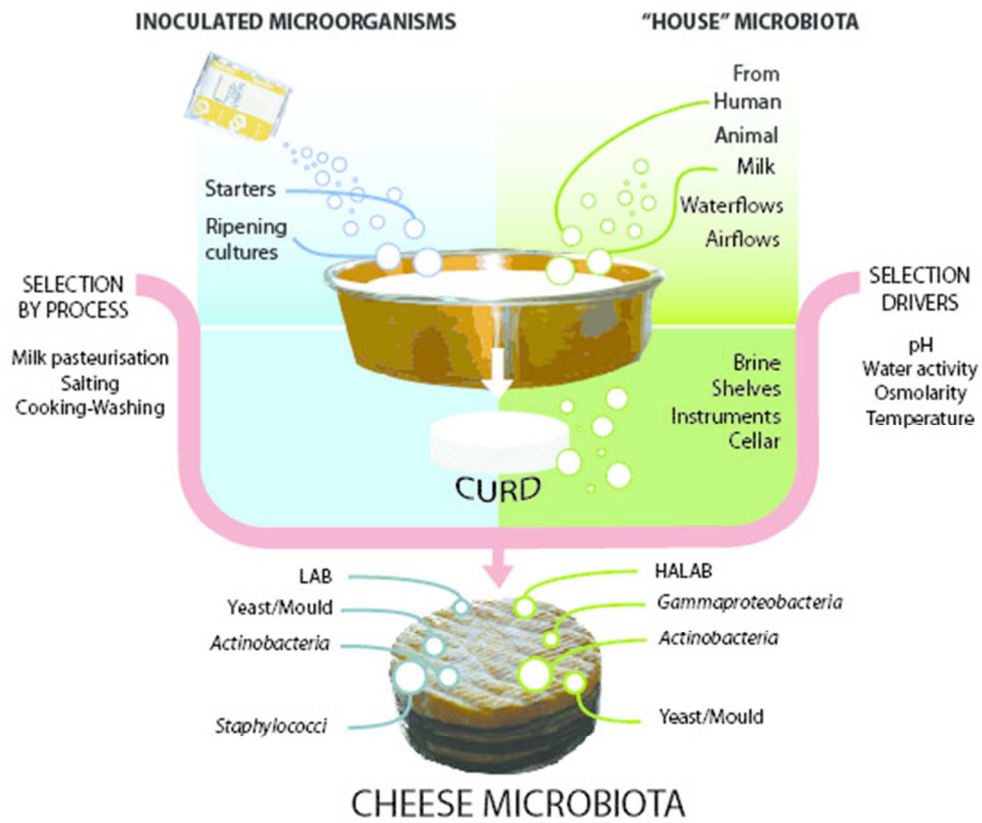
Cheese rind microbial communities: diversity, composition and origin

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3 **1 Cheese rind microbial communities: diversity, composition and origin**

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5 **2 Françoise Irlinger^{1*}, Séverine Layec², Sandra Hélinck², and Eric Dugat-Bony¹**

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52 **12 Abstract**

31 Cheese rinds host a specific microbiota composed of both prokaryotes (such as
32 *Actinobacteria*, *Firmicutes* and *Proteobacteria*) and eukaryotes (primarily yeasts and
33 moulds). By combining modern molecular biology tools with conventional, culture-based
34 techniques, it has now become possible to create a catalogue of the biodiversity that inhabits
35 this special environment. Here, we review the microbial genera detected on the cheese surface
36 and highlight the previously unsuspected importance of non-inoculated microflora - raising
37 the question of the latter's environmental sources and their role in shaping microbial
38 communities. There is now a clear need to revise the current view of the cheese rind
39 ecosystem (*i.e.* that of a well-defined, perfectly controlled ecosystem). Inclusion of these new
40 findings should enable us to better understand the cheese-making process.
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60 **24 Introduction**

53 Cheese is one of the oldest fermented foods created by man. It has been produced and
54 consumed for thousands of years and has been adapted to match the technical, social and
55 economic conditions in various parts of the world. Consequently, the cheese fermentation
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3 28 process is strongly linked to culture and tradition - especially in rural households and village
4 29 communities. Worldwide, there are about 1,000 distinct types of (most artisanal) cheese, with
5 30 a remarkable variety of textures, visual aspects, aromas and flavours. The organoleptic
6 31 richness and diversity of these cheeses (all of which are prepared from the same raw material,
7 32 *i.e.* milk) can be explained by the action of a large number of microorganisms that flourish in
8 33 cheese and that specifically degrade the components of the curd during the cheese-making
9 34 process (Montel *et al.*, 2014).

15 35 The cheese rind's characteristics help to define the type of cheese and largely determine its
16 36 flavour. Furthermore, cheese rind constitutes a man-made ecosystem that has resulted from
17 37 the selection and establishment of functional microbial communities. Cheese rind
18 38 communities are less complex than natural environments, such as soil or the lumen of the
19 39 digestive tract. Moreover, the cheese rind ecosystem is in contact with the external
20 40 environment and consequently differs dramatically from the cheese core in terms of microbial
21 41 composition and biochemical characteristics (Almena-Aliste & Mietton, 2014). The surface
22 42 microflora has a major organoleptic impact on cheese thanks to its various enzymatic
23 43 activities and its role as a barrier against pathogens and spoilage microorganisms (Irlinger &
24 44 Mounier, 2009). Consequently, studying and understanding this particular ecosystem is of
25 45 great interest from both public health and economic perspectives.

34 46 Our knowledge of microbial diversity in cheese rinds is still fragmented but has expanded
35 47 significantly over the last decade, due to the use of molecular biology techniques to profile
36 48 microbial communities. Here, we review and summarize recent literature on the microbial
37 49 diversity of cheese rinds. This analysis enabled us to establish an up-to-date catalogue of the
38 50 microbial taxa associated with this special environment. Furthermore, we draw a distinction
39 51 between technological and adventitious microflora, and focus on the latter's possible origins.
40 52 This evaluation should be of immense help in exploring unstudied cheese environments and
41 53 retrieving isolates belonging to taxa not previously observed on the cheese surface.

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51 **The distribution of bacterial and fungal genera within rind communities**

53 56 The composition of the cheese surface microbiota has been studied for several decades via the
54 57 application of conventional, culture-based analyses (Valdès-Stauber *et al.*, 1997; Maoz *et al.*
55 58 2003; Viljoen *et al.*, 2003; Feurer *et al.*, 2004a; Mounier *et al.*, 2005; Callon *et al.*, 2006;

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3 59 Larpin *et al.*, 2006; Lopandic *et al.*, 2006; Florez & Mayo, 2006; Rea *et al.*, 2007; Goerges *et*
4 60 *al.*, 2008; Mounier *et al.*, 2009; Bleicher *et al.*, 2010; Panelli *et al.*, 2012; Roth *et al.*, 2010;
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6 61 Larpin-Laborde *et al.*, 2011; Amato *et al.*, 2012; Lavoie *et al.*, 2012; Gori *et al.* 2013; Cogan
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8 62 *et al.*, 2014; Gkazionis *et al.*, 2014) and, since the end of the 20th century, by using culture-
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10 63 independent molecular biology methods based on the direct extraction of DNA and/or RNA
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12 64 from the matrix. The first culture-independent methods to be applied to cheese microbial
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14 65 communities were based either on molecular fingerprinting (such as PCR- denaturing gradient
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16 66 gel electrophoresis, PCR- temperature gradient gel electrophoresis and PCR-single strand
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18 67 conformation polymorphism analysis) or cloning and sequencing of target rRNA sequences
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20 68 after PCR amplification (Ercolini *et al.*, 2003; Feurer *et al.*, 2004a; Ogier *et al.*, 2004; Callon
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22 69 *et al.*, 2006; Parayre *et al.*, 2007; Dolci *et al.*, 2009; Mounier *et al.*, 2009; Fontana *et al.* 2010;
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24 70 Roth *et al.*, 2010; Feligni *et al.*, 2012; Dolci *et al.*, 2013; Gori *et al.* 2013, Gkazionis *et al.*,
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26 71 2014; Hermet *et al.*, 2014; Schornsteiner *et al.*, 2014). The recent development of high-
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28 72 throughput sequencing (HTS) technologies has dramatically changed our view of microbial
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30 73 communities inhabiting fermented food products. In particular, metabarcoding analysis (*e.g.*
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32 74 deep sequencing of phylogenetic biomarker amplicons, such as variable regions of rRNA
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34 75 genes or intergenic transcribed spacers amplified using universal PCR primers) now enable
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36 76 microbial communities to be profiled with unprecedented depth (Ercolini 2013; Bokulich and
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38 77 Mills, 2012). To the best of our knowledge, only four publications have described the
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40 78 application of HTS techniques to cheese rind samples (Quigley *et al.*, 2012; Bokulich *et al.*,
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42 79 2013; Wolfe *et al.*, 2014; Delcenserie *et al.*, 2014). However, it is likely that greater use of
43
44 80 HTS techniques in the future will result in large-scale, highly detailed descriptions of the
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46 81 microbial communities from cheese rinds.

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48 82 The literature on the microbial diversity of cheese rinds (studied with both culture-dependent
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50 83 and -independent approaches) is summarized in Tables 1 and 2. Although this list is not
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52 84 exhaustive, it encompasses most of the results generated over the last fifteen years. Four main
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54 85 cheese types have been considered: i) unpressed, uncooked soft cheeses with bloomy or
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56 86 washed rinds, (ii) pressed, uncooked, semi-hard cheeses with natural or washed rinds, (iii)
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58 87 pressed, cooked hard cheeses with washed rinds and (iv) blue-veined cheeses. The production
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60 88 processes for these cheeses differ in terms of three critical steps (namely coagulation, draining
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90 89 and ripening), which means that cheese varieties have very different formats and
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92 90 physicochemical characteristics (Almena-Aliste & Mietton, 2014). Accordingly, a great
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94 91 variety of species assemblages has been observed. The picture that has emerged from the

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3 92 published data is that microbial populations may differ extensively from one cheese variety to
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5 93 another but also within a given variety, as a function of the place of production or the season
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7 94 (Rea *et al.*, 2007; Viljoen *et al.*, 2003). Nonetheless, the specific signature of a cheese surface
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9 95 community is due to the presence of a set of different microbial species, rather than a
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11 96 particular species. Many phylotypes are common to most cheese varieties but have varying
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13 97 populations and levels of complexity. The microbial communities of cheese rinds range from
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15 98 simple to complex assemblages harbouring *Firmicutes*, *Actinobacteria*, *Proteobacteria*,
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17 99 *Bacteroidetes*, yeasts and moulds. These microorganisms vary in terms of their abundance
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19 100 and diversity during the cheese ripening process, depending on the type of rind (bloomy,
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21 101 washed or natural) and the technology used (soft, hard or semi-hard). At the beginning of the
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23 102 cheese-making process, lactic acid bacteria (LAB) from starter cultures are dominant in terms
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25 103 of cell counts. Over the first few days of ripening, yeasts and/or moulds colonize the cheese
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27 104 surface. The viable cell count for yeast reaches around 6-8 log₁₀ cfu/cm² and then remains
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29 105 fairly constant until the end of ripening. The progressive deacidification of the cheese surface
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31 106 by the yeasts and/or moulds favours the establishment of an acid-sensitive, salt-tolerant
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33 107 bacterial community. The final bacterial cell count is 1 to 2 log₁₀ units higher than the yeast
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35 108 cell count (Cogan *et al.*, 2014). After reviewing 33 cheese rind studies, we identified 104
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37 109 bacterial genera (1 *Acidobacteria*, 28 *Actinobacteria*, 5 *Bacteroidetes*, 24 *Firmicutes* and 46
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39 110 *Proteobacteria*) and 39 fungal genera (21 moulds and 18 yeasts). On average, 10.2 bacterial
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41 111 genera (ranging from 3 to 30, depending on the cheese variety) and 4.5 fungal genera (ranging
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43 112 from 1 to 11) were detected in cheese rinds (Tables 1, 2 and 3). We did not take account of
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45 113 the overall diversity studies performed by Wolfe *et al.* (2014) and Quigley *et al.* (2012) using
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47 114 HTS technologies because the types and varieties of cheese were not specified. Among the
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49 115 yeasts, *Debaryomyces*, *Yarrowia*, *Candida* and *Geotrichum* were the most frequently detected
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51 116 genera (present in 86%, 57%, 54% and 49% of the cheeses, respectively), followed by
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53 117 *Kluyveromyces* (32%) and *Pichia* (22%). Of the filamentous fungi, *Penicillium* was most
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55 118 frequently detected (in 19% of the cheeses), followed by *Scopulariopsis* and *Fusarium* (both
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57 119 8%). *Staphylococcus* was the most frequently found *Firmicutes* genus (present in 78% of the
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59 120 cheeses). Other abundant *Firmicutes* were the LAB *Lactococcus*, *Enterococcus*,
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121 *Lactobacillus*, *Streptococcus* and *Vagococcus*, present in 50%, 41%, 25%, 22% and 16% of
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123 the cheeses respectively, and halophilic lactic bacteria such as *Marinilactibacillus* and
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125 *Facklamia* (present in 22% and 16% of the cheeses, respectively). Considering the
Actinobacteria, *Brevibacterium*, *Corynebacterium* and *Arthrobacter* were the most frequent
genera (present in 75%, 75% and 66% of the cheeses, respectively), followed by

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3 126 *Brachybacterium*, *Microbacterium*, *Agrococcus* and *Micrococcus* (present in 38%, 38%, 19%
4 and 19% of the cheeses, respectively). The genera *Psychrobacter*, *Halomonas*,
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6 128 *Pseudoalteromonas* and *Vibrio* (which are all halotolerant *Proteobacteria*) are also major
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8 129 cheese rind microorganisms, since they were detected in 33%, 31%, 22% and 19% of the
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10 130 cheeses, respectively.

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12 131 Similar profiles have been obtained in recent, large-scale HTS studies. For example, Wolfe *et*
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14 132 *al.* (2014) characterized the fungal and bacterial diversity of 137 different cheese rinds
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16 133 collected in the United States and 9 European countries, and Quigley *et al.* (2012) studied
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18 134 bacterial communities from 11 Irish cheese rinds. In Wolfe *et al.*'s study (2014), 14 bacterial
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20 135 and 10 fungal dominant genera (representing >1% of the overall dataset) were distributed
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22 136 across three different rind biofilms (bloomy, washed and natural) with varying abundance. Of
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24 137 these, only two fungal genera (*Aspergillus* and *Sporandonema*) and one bacterial genus
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26 138 (*Nocardiopsis*) were not detected in the other studies shown in Tables 1 and 2. This
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28 139 observation shows that conventional culture-based and culture-independent techniques detect
29
30 140 the same dominant populations as HTS, leading to the conclusion that all dominant
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32 141 microorganisms are cultivable and the selective media used for enumerating and isolation of
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34 142 cheese strains are appropriate and effectively allow the highlighting of the whole rind
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36 143 biodiversity. It is noteworthy that representative isolates from 24 dominant genera detected by
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38 144 sequencing were easily retrieved from dilutions of rind samples (Wolfe *et al.*, 2014). On
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40 145 average, there were 6.5 dominant bacterial genera and 3.2 dominant fungal genera per rind
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42 146 sample (Table 3). These values are slightly lower than those estimated in our review on the
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44 147 basis of either culture-dependent or culture-independent techniques - almost certainly because
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46 148 (i) only dominant genera were considered in the calculation and (ii) Wolfe *et al.* (2014)
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48 149 excluded LAB genera (*Lactococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus*) from
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50 150 their analysis. Indeed, 54 subdominant genera (44 bacteria and 10 fungi) were detected in the
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52 151 study by Wolfe *et al.* (2014) (Table 4), including genera commonly found on the cheese
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54 152 surface such as the eukaryotes *Mucor*, *Kluyveromyces*, *Yarrowia* and *Pichia* and the bacteria
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56 153 *Leucobacter* and *Microbacterium*. Moreover, Wolfe *et al.* (2014) also detected 4 fungal
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58 154 genera and 27 bacterial genera that had never been mentioned in other reports. This finding
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60 155 reflects the powerful ability of HTS technologies to access the diversity of low-abundant taxa
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157 156 for the first time. In terms of LAB (often considered to be cheese curd contaminants), the
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159 157 current literature suggests that these bacteria are indeed found in the cheese rind itself (Table
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158 1). Furthermore, Quigley *et al.* (2012) estimated that LAB taxa account for between 2% and

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3 159 4.8% of the total bacterial community in washed rinds and up to 98% in some natural rinds.
4 160 The reported presence of LAB in cheese rinds may also be due to inter-study differences in
5 161 the sampling technique (i.e. the thickness of the rind samples, or whether the rinds are scraped
6 162 or cut off), since this may result in inadvertent sampling of the cheese core and thus
7 163 contamination of the rind by LAB. By combining the literature results, we found that culture-
8 164 dependent methods and culture-independent molecular biology techniques are
9 165 complementary, rather than contradictory or exclusive). On one hand, culture-based
10 166 techniques are easily able to characterize the individual properties of dominant members of
11 167 the cheese rind microflora (e.g. genomic repertoires, metabolic capacities, and growth
12 168 kinetics). On the other hand, molecular biology methods (especially those based on HTS
13 169 technologies) are valuable for highlighting the existence of previously unexpected
14 170 subdominant populations. In turn, this information will be very helpful in designing specific
15 171 culture media for the isolation of novel members of the cheese microbiota.
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173 **Ripening adjunct cultures versus house microbiota**

174 If we take into account Bourdichon *et al.*'s 2012 inventory of microbial food cultures (an
175 update of the 2002 International Dairy Federation/European Food and Feed Cultures
176 Association list of cultures with "technological beneficial use"), some of the above-mentioned
177 genera may be considered as starters or ripening cultures that are deliberately inoculated to
178 produce cheeses under well-controlled ripening conditions. Thus, the filamentous fungal
179 species that are highly adapted to the cheese surface include *Penicillium camemberti* (a
180 species only found in cheese, derived from *Penicillium commune*), the filamentous yeast
181 *Geotrichum candidum* and *Fusarium domesticum*. These species are commonly used and
182 found in cheeses requiring a velvety appearance, such as Camembert, Brie, Taleggio,
183 Reblochon, Saint-Nectaire, Tilsit, Limburger, Brick and Raclette (Arteau *et al.*, 2010). The
184 species *F. domesticum* (also referred to as "*Anticollanti*" and formerly assigned taxonomically
185 to the *Trichotecium domesticum* species or the *Cylindrocarpon* genus) is able to favour drying
186 of the cheese surface and thus a reduction in the latter's stickiness (Bachmann *et al.*, 2005;
187 Ropars *et al.*, 2012). The presence of *G. candidum* (an anamorph of *Galactomyces candidus*)
188 does not necessarily result from deliberate inoculation. Indeed, *G. candidum* is known to be
189 ubiquitous and appears rapidly on the cheese surface, regardless of geographical distribution
190 or the type of technology (Wolfe *et al.*, 2014). Nevertheless, *G. candidum*'s growth is

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3 191 inhibited in cheeses with a high salt content (Boutrou & Gueguen, 2005). *Penicillium*
4 192 *roqueforti* is a major ripening species in blue-veined cheeses such as Roquefort, Gorgonzola,
5 193 Stilton, Danish Blue and Cabrales. In Stilton, it has been shown that *P. roqueforti* grows
6 194 within the veins and not on the surface (Gkatzionis *et al.*, 2014). *Scopulariopsis flava* is
7 195 abundant in the washed rinds of Danish Harvati cheese, Swiss Tilsit, Austrian Vorarlberger
8 196 Bergkäse hard cheeses, and French tommes from the Pyrenees and Ossau-Iraty (Amato *et al.*,
9 197 2012; Gori *et al.*, 2013; Schornsteiner *et al.*, 2014; Ropars *et al.*, 2012). It is also used as a
10 198 ripening culture (Bourdichon *et al.*, 2012; Hermet *et al.*, 2012; Ropars *et al.*, 2012). Wolfe *et*
11 199 *al.* (2014) also observed that members of the *Scopulariopsis* genus are frequently detected in
12 200 dry natural rinds and that the genus' presence is negatively correlated with surface moisture
13 201 levels. Several *Mucor* species (*M. mucedo*, *M. plumbeus* and *M. racemosus*) can be used as
14 202 ripening cultures in some cheeses with washed or natural rinds, including Saint-Nectaire,
15 203 Tomme de Savoie and Taleggio (Barrios *et al.*, 1998; Hermet *et al.*, 2012). The most common
16 204 commercial ripening yeasts are *Kluyveromyces lactis*, *Kluyveromyces marxianus* and
17 205 *Debaryomyces hansenii* (Lavoie *et al.*, 2012). Given that *D. hansenii* is ubiquitous and highly
18 206 salt tolerant, it may be present at the surface of most cheese varieties - even in cases in which
19 207 it has not been added deliberately. This species is very common in blue-veined cheeses but is
20 208 also found in washed and bloomy rinds (Gkatzionis *et al.*, 2014; Schornsteiner *et al.*, 2014;
21 209 Cocolin *et al.*, 2009; Valdès-Stauber *et al.*, 1997). *Kluyveromyces lactis* and *K. marxianus* are
22 210 involved in the very early steps of ripening (thanks to their ability to ferment lactose) but
23 211 generally disappear after a few days of ripening (Arteau *et al.*, 2010; Larpin *et al.*, 2006;
24 212 2011).

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40 213 When considering the *Firmicutes*, the LAB *Lactococcus lactis* ssp. *lactis* and *L. lactis* ssp.
41 214 *cremoris* are the major components of the mesophilic lactic starter cultures widely used in
42 215 cheese-making. They are frequently combined with other mesophilic (*Leuconostoc*
43 216 *mesenteroides*, *L. pseudomesenteroides*) or thermophilic (*Streptococcus thermophilus*) LAB.
44 217 The non-starter LAB (NSLAB) mainly correspond to facultative heterofermentative
45 218 lactobacilli (*Lactobacillus paracasei*, *L. casei*, *L. rhamnosus*, *L. plantarum*, *L. curvatus*, and
46 219 *Pediococcus acidilactici*). In some technologies, NSLAB are added as adjunct cultures to
47 220 accelerate the ripening process; they help to produce the free amino acids that improve cheese
48 221 flavour and reduce harshness and bitterness. Beside LAB, other components of commercial
49 222 cheese-making cultures include *Propionibacterium freudenreichii* (an anaerobic
50 223 actinobacterium involved in flavour and eye formation in Swiss-type cheeses), coagulase-

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3 224 negative staphylococci (mostly *S. xylosus* and *S. equorum*) and other aerobic *Actinobacteria*
4 225 (*Brevibacterium aurantiacum*, *Brevibacterium linens*, *Arthrobacter arilaitensis*, *Arthrobacter*
5 226 *sp.*, *Corynebacterium casei*, *Corynebacterium variabile*, *Brachybacterium alimentarium* and
6 227 *Microbacterium gubbeenense*) that contribute to the flavour and colour of surface-ripened
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8 228 cheeses (Bockelmann, 2010). The only Gram-negative bacterium used in commercial cultures
9 229 is the enterobacterium *Hafnia alvei*. However, this species can also be adventitious. When *H.*
10 230 *alvei* is inoculated into smear-ripened or mould-ripened cheeses manufactured with
11 231 pasteurized milk, it increases the level of volatile sulphur compounds and therefore improves
12 232 the cheese flavour (Irlinger *et al.*, 2012).

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19 233 It is noteworthy that the strains from commercial ripening cultures used for the manufacturing
20 234 of cheeses made from pasteurized milk are not necessarily found to be the dominant surface
21 235 microorganisms (Feurer *et al.*, 2004b; Goerges *et al.*, 2008; Larpin *et al.*, 2011; Rea *et al.*,
22 236 2007; Gori *et al.*, 2013). For example, the species *Brevibacterium aurantiacum* (formerly
23 237 assigned to *B. linens* and frequently used for the manufacturing of smear-ripened cheeses) is
24 238 only sporadically found on the surface of these cheeses (Larpin-Laborde *et al.*, 2011; Goerges
25 239 *et al.*, 2008; Mounier *et al.*, 2005; Rea *et al.*, 2007; Brennan *et al.*, 2002). According to most
26 240 of the studies considered in Tables 1 and 2, the cheese surface is dominated by adventitious
27 241 microflora and by microorganisms from starter and secondary cultures. In a study of the
28 242 microbial diversity of 137 cheese rind communities (Wolfe *et al.* 2014), it was estimated that
29 243 60% of the bacterial genera and 25% of the fungal genera detected did not originate from
30 244 starter or secondary cultures. Many of the “house” flora cited in Tables 1 and 2 correspond to
31 245 environmental genera, such as marine halotolerant *Proteobacteria* (*Psychrobacter*,
32 246 *Pseudoalteromonas*, *Halomonas*, *Vibrio* and *Advenella*), and marine halophilic and
33 247 alkaliphilic LAB (*Vagococcus*, *Facklamia* and *Marinilactibacillus*) (Wolfe *et al.*, 2014;
34 248 Quigley *et al.*, 2012; Schornsteiner *et al.*, 2014; Ishikawa *et al.*, 2007; Amato *et al.*, 2012;
35 249 Mounier *et al.*, 2009; Roth *et al.*, 2011; Bokulich *et al.*, 2013; Bleicher *et al.*, 2010; Gori *et al.*,
36 250 2013).

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49 251 Among the yeasts, *Yarrowia lipolytica* is ubiquitous and grows spontaneously on cheese
50 252 surfaces. It has been shown that other dairy yeast species (including *D. hansenii* and *G.*
51 253 *candidum*) can be outgrown by *Y. lipolytica* (Mounier *et al.*, 2008; Viljoen *et al.*, 2003).
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53 254 Several species of *Candida* and *Pichia* are also frequently found on the rind of European
54 255 smear-ripened cheeses (Larpin *et al.*, 2006, 2011; Mounier *et al.*, 2009; Cogan *et al.*, 2014).
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56 256 Some species have been detected only in specific rinds. For example, members of the *Yaniella*
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3 257 genus have been detected in Swiss and Austrian washed rinds (Schornsteiner *et al.*, 2014;
4 258 Wolfe *et al.*, 2014). The fungal airborne genus *Cladosporium* is commonly detected in the rind
5 259 of blue-veined cheeses (*e.g.* Taleggio) and the rind of mould-ripened cheeses (*e.g.*
6 260 Camembert) (Panelli *et al.*, 2012, Arteau *et al.*, 2010).

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10 261 Furthermore, several studies have shown that the bacterial flora dominating cheese surfaces
11 262 belong to well-known inoculated species assigned to genera *Staphylococcus*,
12 263 *Corynebacterium*, *Brachybacterium*, *Arthrobacter*, *Microbacterium*, *Brevibacterium*,
13 264 *Debaryomyces* and *Geotrichum*, but that the dominating strains tend to originate from the
14 265 "house" microflora rather than the added ripening culture (Gori *et al.*, 2013; Mounier *et al.*,
15 266 2006; Goerges *et al.*, 2008; Rea *et al.*, 2007; Feurer *et al.*, 2004b). These findings emphasize
16 267 that (i) the microbial composition of cheese rind is strongly influenced by the environmental
17 268 communities present in the area of cheese production and (ii) microorganisms selected as
18 269 ripening cultures for their expression of defined technological functions often behave
19 270 differently in complex microbial communities, probably due to their poor adaptation to
20 271 cheese-making processes and their lack of competitive advantage over indigenous microbiota.
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33 273 **Reservoirs of microbial diversity**

34 274 The presence of adventitious microorganisms in rind raises several questions: (i) how do they
35 275 colonize the cheese-making environment, (ii) how do they transfer to the cheese surface and
36 276 (iii) which technological parameters favour or hamper their development? Adventitious
37 277 microorganisms may be present through the entire production chain, from the farm to the final
38 278 product. This is especially true of dairy environments such as raw milk, dairy utensils (*e.g.*
39 279 wooden shelves, vat or brines) and the atmosphere of farms or the ripening cellar - all of
40 280 which act as potential vectors (Figure 1). Although many studies have focused on microbial
41 281 diversity in raw milk (reviewed in Quigley *et al.*, 2013), few studies have looked at the impact
42 282 of cheese-processing environments on microbial community assemblages in specific
43 283 production units. However, all the available studies show that each manufacturing unit has a
44 284 specific house microflora, which is dependent on the environmental conditions prevailing
45 285 during the cheese-making and is characterized by a typical, stable and recurrent microbiota
46 286 that drives the ripening and potentially has a role in the development of the cheese's
47 287 organoleptic properties (Feligini *et al.*, 2012; Mounier *et al.*, 2006; Viljoen *et al.*, 2003;
48 288 Panelli *et al.*, 2012; Bokulich *et al.*, 2013; Schirmer *et al.*, 2013). The microbial diversity in
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3 289 raw milk is substantial, since a single milk sample can contain as many as 36 dominant
4 290 microbial species (for a review, see Montel *et al.* (2014)). The microbial diversity in milk
5 291 varies from farm to farm and is strongly influenced by the overall farm management system.
6 292 The teat surface is the main source of useful cheese-making bacteria present in milk
7 293 (Vacheyrou *et al.*, 2011; Verdier Metz *et al.*, 2012). The fungal species most frequently
8 294 present in raw cow's milk sampled at different locations and different farms have been
9 295 assigned to *Penicillium commune*, *Yarrowia lipolytica*, *Debaryomyces hansenii*,
10 296 *Kluyveromyces marxianus* and several species of *Candida* (Panelli *et al.*, 2013; Lavoie *et al.*,
11 297 2012; Vacheyrou *et al.*, 2011; Fleet, 2007; Viljoen *et al.*, 2003). While no significant
12 298 geographical influence was established, there is a difference in microbiota composition
13 299 between farm milk and the dairy tank milk. The former is dominated by Gram-positive
14 300 bacteria (*Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Kocuria*, *Lactococcus*,
15 301 *Lactobacillus* and *Enterococcus*), and the latter is dominated by Gram-negative bacteria
16 302 (*Pseudomonas*, *Acinetobacter*, *Chryseobacterium*, *Achromobacter*, *Halomonas* and
17 303 *Psychrobacter*) (Fricker *et al.*, 2011; Vacheyrou *et al.*, 2011; Delbes *et al.*, 2007; Raats *et al.*,
18 304 2011; Rasolofso *et al.*, 2010). This disparity may be attributed to differences in storage
19 305 temperature and storage time. During cheese-making, the main shifts in rind microbial
20 306 composition occurred during curd production and ripening. This observation shows that the
21 307 two key driving forces for microbial growth are pH (which decreases during curd production
22 308 and increases at mid-ripening) and the salt content (Figure 1). These physical-chemical
23 309 attributes exert selective pressure on the microbiota and consequently favour microbial
24 310 species that are specifically adapted to these environmental constraints (Irlinger & Mounier,
25 311 2009). For example, the deacidification of the cheese surface (due to the consumption of
26 312 lactate and the production of ammonia by moulds and yeasts) stimulates the growth of acid-
27 313 sensitive aerobic bacteria, such as *Actinobacteria* and *Proteobacteria*. Although there are too
28 314 few data on the presence of strains related to alkaliphilic LAB (*e.g.* *Marinilactibacillus*,
29 315 *Vagococcus*, *Facklamia*) to form an opinion on the latter's practical significance and possible
30 316 origin, the environmental study by Bokulich *et al.*, (2013) has shown that γ -*Proteobacteria*
31 317 (*e.g.* *Psychrobacter*, *Pseudoalteromonas*, *Halomonas*, and *Vibrio*) present in cheese rinds also
32 318 dominated the washed-rind maturation room surfaces (*i.e.* the wash bucket, draining table,
33 319 brine tank, aging rack and drains) in one of the two dairy facilities studied. These genera are
34 320 known to be halophilic (or halotolerant) and psychrotolerant, which could explain their
35 321 ubiquitous distribution in food products (cheese, meat and fish) and in natural milieus such as
36 322 sea water and soil (Nychas *et al.*, 2008, Olofson *et al.*, 2007, Margesin *et al.*, 2011). In this

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3 323 respect, the salting step (which consists in rubbing salt on the cheese surface, or soaking
4 324 cheeses in a brine solution) generates a high salt concentration that favours the growth of
5 325 these microorganisms or other halotolerant microorganisms such as *Staphylococcus*,
6 326 *Brevibacterium*, *Arthrobacter*, *Corynebacterium* and *Leucobacter* (Ishikawa *et al.*, 2006;
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8 327 Mounier *et al.*, 2005; Mounier *et al.*, 2006; Bokulich *et al.*, 2013; Cogan *et al.*, 2014; Feligini
9
10 328 *et al.*, 2012). Wooden processing surfaces (including aging shelves and vats) are also rich
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12 329 sources of microorganisms, which generally produce biofilms that are stable over several
13
14 330 seasons. These biofilms are dominated by yeasts, moulds and *Actinobacteria*, and correlations
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16 331 have been observed with the microbial groups in cheese rinds (Mounier *et al.*, 2006; Mariani
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18 332 *et al.*, 2007; Soares *et al.*, 2011; Didienne *et al.*, 2012).
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21 22 334 **Conclusions and future perspectives**

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24 335 Thanks to efforts over the past decade, we are starting to obtain a reliable, in-depth picture of
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26 336 the microbial diversity of cheese rinds. In this review, we emphasized that the cheese rind
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28 337 environment hosts a variety of microorganisms from several sources (including commercial
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30 338 ripening adjunct cultures and adventitious flora). Indeed, cheese production and aging
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32 339 environments may be a rich source of microbes throughout the course of fermentation.
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34 340 However, little attention has been paid to the characterization of facility-specific “house”
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36 341 microbiota and how the latter are selected, survive, and colonise cheese rinds. This is a topic
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38 342 that needs to be explored further.

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40 343 We have summarized the literature data at the genus level, and have thus shed light on a
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42 344 common set of a dozen genera distributed across many cheese varieties. These findings
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44 345 suggest the existence of a core microbiota that has adapted to the cheese surface. However,
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46 346 large-scale studies of phylotypes at the species or strain level are now required. Recent
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48 347 advances in HTS technologies have made this type of study feasible - providing that similar
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50 348 progress in the tools' taxonomic resolution can also be achieved. Given that HTS and cloning-
51
52 349 dependent approaches are only semi-quantitative, it might also be interesting to use
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54 350 quantitative PCR to more precisely quantify the abundance of certain phylotypes. Lastly,
55
56 351 there is a need to focus on the species' ecological adaptation, colonization capabilities, ability
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58 352 to resist and/or adapt to disturbances (e.g. environmental changes, or contamination by a
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60 353 pathogen) and mutual interactions. In particular, the food industry is looking for powerful

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354 tools that enable the in-depth, reliable characterization of food microbial communities and
355 thus provides better control of production processes.

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3 361 **Table 1.** The presence of bacterial genera in cheese rinds, as determined in selected
4 362 biodiversity studies¹

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6 363 A total of 77 bacterial genera are listed (1 *Acidobacteria*, 18 *Actinobacteria*, 3 *Bacteroidetes*, 19
7 364 *Firmicutes* and 36 *Proteobacteria*).

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9 365 ¹ The bibliographic references for the data in this table are as follows: Cogan *et al.* (2014); Goerges *et*
10 366 *al.* (2008); Valdès-Stauber *et al.* (1997); Larpin *et al.* (2006); Larpin-Laborde *et al.* (2011); Mounier *et*
11 367 *al.* (2009); Mounier *et al.* (2005); Ogier *et al.* (2004); Feurer *et al.* (2004); Bleicher *et al.* (2010);
12 368 Maoz *et al.* (2003); Parayre *et al.* (2007); Rea *et al.* (2007); Gori *et al.* (2013); Fontana *et al.* (2010);
13 369 Feligni *et al.* (2012); Bockulich *et al.* (2013); Amato *et al.* (2012); Roth *et al.* (2010); Dolci *et al.*
14 370 (2009); Dolci *et al.* (2013); Schornsteiner *et al.* (2014); Ercolini *et al.* (2003); Quigley *et al.* (2012);
15 371 Wolfe *et al.* (2014) and Delcenserie *et al.*, (2014).

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18 372 ² Various methods were used to detect dominant genera in cheese rind samples:

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20 373 • Culture-dependent (CD) methods (traditional plating and isolation of bacteria, followed by
21 374 genotyping).

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23 375 • Culture-independent (CI) methods (direct extraction of microbial DNA from cheese rinds, followed
24 376 by identification via molecular biology methods such as PCR-DGGE/TGGE, PCR-CE-SSCP, and 16S
25 377 rDNA cloning/sequencing.

26
27 378 • High-throughput sequencing (HTS, direct extraction of microbial DNA from cheese rinds, followed
28 379 by identification via V4/V1-V3 16S rDNA amplicon sequencing (454 pyrosequencing or Illumina)).

29
30 380 ³ The frequency calculation takes account of 31 different cheese varieties from all separate studies
31 381 except Quigley *et al.*, 2012 and Wolfe *et al.*, 2014.

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33 382 ⁴ The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et*
34 383 *al.*, 2014).

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36 384 ⁵ *Acidobacteria*

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38 385 ⁶ *Bacteroidetes*

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43 387 **Table 2.** The presence of fungal genera in cheese rinds, as determined in selected biodiversity
44 388 studies¹

45
46 389 A total of 35 fungal genera are listed (17 molds and 18 yeasts)

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48 390 ¹ The bibliographic references for the data in this table are as follows: Cogan *et al.* (2014), Goerges *et*
49 391 *al.* (2008), Valdès-Stauber *et al.* (1997), Larpin *et al.* (2006), Mounier *et al.* (2009), Mounier *et al.*
50 392 (2005), Hermet *et al.* (2014), Gori *et al.* (2013); Lopandic *et al.* (2006), Panelli *et al.* (2010), Viljoen *et*
51 393 *al.* (2003), Lavoie *et al.* (2012), Bockulich *et al.* (2013), Callon *et al.* (2006), Amato *et al.* (2012),
52 394 Dolci *et al.* (2009), Schornsteiner *et al.* (2014), Gkazonis *et al.* (2014), Florez & Mayo (2007) and
53 395 Wolfe *et al.* (2014).

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56 396 ² Various methods were used to detect dominant genera in cheese rind samples:

397 • Culture-dependent (CD) methods (traditional plating and isolation of fungi, followed by genotyping).
 398 • Culture-independent (CI) methods (direct extraction of microbial DNA from cheese rinds, followed
 399 by identification via molecular biology methods such as PCR-DGGE/TGGE, PCR-CE-SSCP, and 18S
 400 rDNA cloning/sequencing;

401 • High-throughput sequencing (HTS, direct extraction of microbial DNA from cheese rinds, followed
 402 by identification via internal transcribed spacers amplicon sequencing (454 pyrosequencing or
 403 Illumina)).

404 ³ The frequency calculation takes account of 33 different cheese varieties from 19 separate studies.

405 ⁴ The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et*
 406 *al.*, 2014).

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408 **Table 3.** Comparison of genus richness in different types of cheeses, using culture-dependent
 409 and culture-independent methods.

410 nd: not determined.

411 +X: number of genera listed as being subdominant (*i.e.* abundance <1%)

412 DGGE: denaturing gradient gel electrophoresis

413 TGGE: temperature gradient gel electrophoresis

414 SSCP: single strand conformation polymorphism

415 ITS: intergenic transcribed spacers

416 HTS: high-throughput sequencing

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418 **Table 4.** The subdominant bacterial and fungal genera in cheese rinds, as determined by an HTS study
 419 of 137 European and American cheeses (Wolfe *et al.*, 2014).

420 ¹*Bacteroidetes*

421 ² The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et*
 422 *al.*, 2014)

423 A total of 44 subdominant bacterial genera (15 *Actinobacteria*, 2 *Bacteroidetes*, 6 *Firmicutes* and 21
 424 *Proteobacteria*) and 10 subdominant fungal genera (6 moulds and 4 yeasts) were detected in the 137
 425 cheese rinds

426 * A total of 23 genera (5 *Actinobacteria*, 1 *Firmicute*, 11 *Proteobacteria*, 2 moulds and 4 yeasts) were
 427 found to be dominant in other biodiversity studies.

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5 430 **Figure 1.** Schematic overview of potential reservoirs of the different microbial groups
6 431 colonising cheese rinds.

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8 432 LAB, lactic acid bacteria;

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10 433 HALAB, halophilic and alkalophilic LAB;

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437 **Table 2.** The presence of fungal genera in cheese rinds, as determined in selected biodiversity
 438 studies¹

Types of cheese	soft																	semi-hard					hard		blue			soft	Frequency (%) ³	Frequency (%) ⁴							
	Cheese variety																																				
Methods ²	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD	CI	CI	CI	CI	CI	CI	CD	CD	CD	CD	HTS	HTS							
Molds																															5	7					
<i>Acromonium</i>																															3						
<i>Alternaria</i>																																3					
<i>Arachnomyces</i>																																3					
<i>Aspergillus</i>																																0	15				
<i>Botryotinia</i>																																3					
<i>Chrysosporium</i>																																0	7				
<i>Cladosporium</i>																																8					
<i>Fusarium</i>																																8	19				
<i>Eutypella</i>																																3					
<i>Mucor</i>																																5					
<i>Nectria</i>																																3					
<i>Penicillium</i>																																19	31				
<i>Peyronellaea</i>																																3					
<i>Pyxidiophora</i>																																3					
<i>Scopulariopsis</i>																																8	43				
<i>Sporandomena</i>																																0	4				
<i>Sporopachydermia</i>																																3					
Yeasts																															54	15					
<i>Candida</i>																																8					
<i>Clavispora</i>																																5					
<i>Cryptococcus</i>																																5					
<i>Debaryomyces</i>																																86	57				
<i>Geotrichum</i>																																49	35				
<i>Issatchenkia</i>																																5					
<i>Kazachstania</i>																																3					
<i>Kluyveromyces</i>																																32					
<i>Metschnikowia</i>																																3					
<i>Pichia</i>																																22					
<i>Rhodotorula</i>																																8					
<i>Saccharomyces</i>																																14					
<i>Starmerella</i>																																3					
<i>Torulasporea</i>																																11					
<i>Trichosporon</i>																																11					
<i>Yarrowia</i>																																57					
<i>Zygosaccharomyces</i>																																3					
<i>Unidentified</i>																																11					
Number of genera	4	5	4	6	7	6	3	5	1	3	3	2	3	2	4	3	5	5	7	10	4	5	4	11	4	5	5	9	2	6	4	5	2	4	4	7	10

439 A total of 35 fungal genera are listed (17 molds and 18 yeasts)
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441 ¹ The bibliographic references for the data in this table are as follows: Cogan *et al.* (2014), Goerges *et al.* (2008), Valdès-Stauber *et al.* (1997), Larpin *et al.* (2006), Mounier *et al.* (2009), Mounier *et al.* (2005), Hermet *et al.* (2014), Gori *et al.* (2013); Lopandic *et al.* (2006), Panelli *et al.* (2010), Viljoen *et al.* (2003), Lavoie *et al.* (2012), Bockulich *et al.* (2013), Callon *et al.* (2006), Amato *et al.* (2012), Dolci *et al.* (2009), Schornsteiner *et al.* (2014), Gkazonis *et al.* (2014), Florez & Mayo (2007) and Wolfe *et al.* (2014).

447 ² Various methods were used to detect dominant genera in cheese rind samples:

- 448 • Culture-dependent (CD) methods (traditional plating and isolation of fungi, followed by genotyping).
- 449 • Culture-independent (CI) methods (direct extraction of microbial DNA from cheese rinds, followed
- 450 by identification via molecular biology methods such as PCR-DGGE/TGGE, PCR-CE-SSCP, and 18S
- 451 rDNA cloning/sequencing;

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3 452 • High-throughput sequencing (HTS, direct extraction of microbial DNA from cheese rinds, followed
4 453 by identification via internal transcribed spacers amplicon sequencing (454 pyrosequencing or
5 454 Illumina)).

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7 455 ³ The frequency calculation takes account of 33 different cheese varieties from 19 separate studies.

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9 456 ⁴ The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et*
10 457 *al.*, 2014).

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460 **Table 3.** Comparison of genus richness in different types of cheeses, using culture-dependent and culture-independent methods.

Number of fungal genera found by:	Type and number (n) of cheeses	Molds	Yeasts	Total	Average value of Richness index	
Culture-dependent method (CD)	Soft cheeses n = 18	7	15	22	4.5	
	Semi-hard cheeses n = 4	2	12	14	6.25	
	Blue cheeses n = 3	2	6	8	3.3	
	Total CD	9	17	26	4.6	
Culture-independent method (CI) PCR-DGGE. TGGE. SSCP / Cloning	Soft cheeses n = 4	1	3	4	2.8	
	Semi-hard cheeses n = 3	3	8	11	6,0	
	Hard cheeses n = 2	5	3	8	4,0	
	Blue cheeses n = 2	1	5	6	4.5	
	Total CI	7	10	17	4.2	
Total (CD + CI)	11	18	29	4.5		
Culture-independent method (HTS) HTS - ITS amplicon sequencing	Soft cheeses n = 2	5	2	7	5.5	
	cheese rinds n = 137	7 (+6)	3 (+4)	10 (+10)	3.2 (nd)	
	Total HTS	7 (+8)	4 (+4)	11 (+12)		
Total (CD + CI + HTS)		17 (+4)	18	35 (+4)		

Number of bacterial genera found by:	Type and number (n) of cheeses	<i>Acidobacteria</i>	<i>Actinobacteria</i>	<i>Firmicutes</i>	<i>Proteobacteria</i>	<i>Bacteroidete</i>	Total	Average value of Richness index	
Culture-dependent method (CD)	Soft cheeses n = 13			12	10	18	40	8.6	
	Semi-hard cheeses n = 3			9	8	6	23	12.7	
	Total CD			15	12	20	47	9.4	
Culture-independent method (CI) PCR-DGGE. TGGE. SSCP / Cloning	Soft cheeses n = 7			7	9	9	25	7.7	
	Semi-hard cheeses n = 4			6	8	2	16	8.0	
	Hard cheeses n = 1			10	4	6	22	22.0	
	Blue cheeses n = 2			1	5	1	7	4.0	
	Total CI			12	13	14	2	41	8.3
Total (CD + CI)			17	15	26	2	60	8.8	
Culture-independent method (HTS) HTS - 16S rDNA amplicon sequencing	Soft cheeses n = 4	1		5	13	16	37	24.5	
	Irish cheese rinds n = 11			4 (nd)	7 (nd)	8 (nd)	19 (nd)	nd	
	cheese rinds n = 137			6 (+15)	1 (+6)	6 (+21)	1 (+2)	14 (+44)	6.5 (nd)
	Total HTS	1		6 (+10)	8 (+7)	8 (+19)	2 (+2)	63	
Total (CD + CI + HTS)		1		18 (+10)	19 (+5)	36 (+10)	3 (+2)	77 (+27)	

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462 nd: not determined.

463 +X: number of genera listed as being subdominant (*i.e.* abundance <1%)

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5 464 DGGE: denaturing gradient gel electrophoresis
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7 465 TGGE: temperature gradient gel electrophoresis
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9 466 SSCP: single strand conformation polymorphism
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11 467 ITS: intergenic transcribed spacers
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13 468 HTS: high-throughput sequencing
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471 **Table 4.** The subdominant bacterial and fungal genera in cheese rinds, as determined by an HTS study
 472 of 137 European and American cheeses (Wolfe *et al.*, 2014).

		Frequency (%) ⁴			Frequency (%) ⁴
<i>Actinobacteria</i>	<i>Actinomyces</i>	0.3	Molds	<i>Eremascus</i>	0.6
	<i>Agrococcus</i> *	6.4		<i>Ilyonectria</i>	3.3
	<i>Agromyces</i>	14.1		<i>Mucor</i> *	5.2
	<i>Amycolatopsis</i>	0.8		<i>Nectria</i> *	2.8
	<i>Citricoccus</i> *	0.6		<i>Oidiodendron</i>	0.8
	<i>Dermacoccus</i>	0.8		<i>Trichotecium</i>	1.7
	<i>Janibacter</i>	1.7	yeasts	<i>Cryptococcus</i> *	<0.1
	<i>Kocuria</i> *	5.2		<i>Kluyveromyces</i> *	3.3
	<i>Leucobacter</i> *	1.1		<i>Trichosporon</i> *	1.1
	<i>Microbacterium</i> *	22.9		<i>Yarrowia</i> *	1.9
	<i>Nesterenkonia</i>	1.7		Number of genera	10
	<i>Promicromonospora</i>	0.3			
	<i>Pseudoclavibacter</i>	1.1			
	<i>Renibacterium</i>	1.7			
	<i>Streptomyces</i>	2.2			
<i>B¹</i>	<i>Chryseobacterium</i>	0.6			
	<i>Myroides</i>	0.8			
<i>Firmicutes</i>	<i>Bacillus</i>	3.0			
	<i>Jeotgalicoccus</i>	2.5			
	<i>Macrococcus</i> *	0.3			
	<i>Oerskovia</i>	0.8			
	<i>Peptoniphilus</i>	0.8			
	<i>Peptostreptococcus</i>	0.6			
<i>Proteobacteria</i>	<i>Alcaligenes</i> *	1.7			
	<i>Averyella</i>	8.6			
	<i>Bordetella</i>	0.3			
	<i>Buttiauxella</i>	0.3			
	<i>Citrobacter</i> *	0.8			
	<i>Cobetia</i> *	4.7			
	<i>Chromohalobacter</i>	0.3			
	<i>Enterobacter</i> *	0.6			
	<i>Haerehalobacter</i>	0.3			
	<i>Idiomarina</i> *	0.3			
	<i>Ignatzschineria</i>	0.8			
	<i>Lysobacter</i>	1.7			
	<i>Marinobacter</i> *	6.9			
	<i>Marinomonas</i> *	3.3			
	<i>Morganella</i> *	8.6			
	<i>Oceanisphaera</i>	0.6			
	<i>Providencia</i> *	1.4			
	<i>Raoultella</i> *	4.1			
<i>Stenotrophomonas</i> *	0.6				
<i>Tatumella</i>	0.3				
<i>Xenorhabdus</i>	0.3				
	Number of genera	44			

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474 ¹*Bacteroidetes*

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3 475 ²The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et*
4 476 *al.*, 2014)

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6 477 A total of 44 subdominant bacterial genera (15 *Actinobacteria*, 2 *Bacteroidetes*, 6 *Firmicutes* and 21
7 478 *Proteobacteria*) and 10 subdominant fungal genera (6 moulds and 4 yeasts) were detected in the 137
8 479 cheese rinds

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10 480 * A total of 23 genera (5 *Actinobacteria*, 1 *Firmicute*, 11 *Proteobacteria*, 2 moulds and 4 yeasts) were
11 481 found to be dominant in other biodiversity studies.

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For Peer Review

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Table 1. The presence of bacterial genera in cheese rinds, as determined in selected biodiversity

Types of cheese		soft															
Cheese variety		German washed rind cheese : Limburger	German washed rind cheese : Romadur	German washed rind cheese : Weinkäse	German washed rind cheese : Harzer	French washed rind cheese : Reblochon	French washed rind cheese : Livarot	French washed rind cheese : Epoisses	French washed rind Munster- type cheese	Irish washed rind cheese : Gubbeen	Irish washed rind cheese : Ardahan	Irish washed rind cheese : Durrus	Irish washed rind cheese : Milleens	Danish washed rind Harvarti type cheese	Danish washed rind Danbo type cheese	Italian natural mold rind cheese : Scimudin	Italian washed rind PDO cheese : Taleggio
	Methods ²	CD	CD	CD	CD	CD	CI	CI	CD	CI	CD	CD	CD	CD	CI	CD	CI
<i>A⁵</i>	<i>Candidatus solibacter</i>																
<i>Actinobacteria</i>	<i>Agrococcus</i>																
	<i>Arcanobacterium</i>																
	<i>Arthrobacter</i>																
	<i>Brachybacterium</i>																
	<i>Brevibacterium</i>																
	<i>Citricoccus</i>																
	<i>Corynebacterium</i>																
	<i>Curtobacterium</i>																
	<i>Kocuria</i>																
	<i>Leucobacter</i>																
	<i>Microbacterium</i>																
	<i>Micrococcus</i>																
	<i>Mycetocola</i>																
	<i>Nocardiopsis</i>																
	<i>Propionibacterium</i>																
	<i>Pseudoclavibacter</i>																
<i>Rothia</i>																	
<i>Yaniella</i>																	
<i>B⁶</i>	<i>Prevotella</i>																
	<i>Psychroflexus</i>																
	<i>Sphingobacterium</i>																
<i>Acidobacteria</i>	<i>Alkalibacterium</i>																
	<i>Atopostipes</i>																
	<i>Bacillus</i>																
	<i>Bavariicoccus</i>																
	<i>Carnobacterium</i>																
	<i>Enterococcus</i>																
	<i>Facklamia</i>																
	<i>Geobacillus</i>																
	<i>Lactobacillus</i>																
	<i>Lactococcus</i>																

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3 A total of 77 bacterial genera are listed (1 Acidobacteria, 18 Actinobacteria, 3 Bacteroidetes, 19 Firmicutes and

4 ¹ The bibliographic references for the data in this table are as follows: Cogan *et al.* (2014); Goerges *et al.* (20

5 ² Various methods were used to detect dominant genera in cheese rind samples:

- 6
7 • Culture-dependent (CD) methods (traditional plating and isolation of bacteria, followed by genotyping).
8 • Culture-independent (CI) methods (direct extraction of microbial DNA from cheese rinds, followed by ident
9 • High-throughput sequencing (HTS, direct extraction of microbial DNA from cheese rinds, followed by ident

10 ³ The frequency calculation takes account of 31 different cheese varieties from all separate studies except Qui

11 ⁴ The frequency calculation takes account of 137 different cheese rinds from a single study (Wolfe *et al.*, 201

12 ⁵ *Acidobacteria*

13 ⁶ *Bacteroidetes*
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For Peer Review

7 studies¹

	semi-hard						hard	blue		soft						
	Austrian long-ripened washed rind Tilsit	Swiss long-ripened washed rind Tilsit	Swiss raclette type cheese	Swiss raclette type cheese	Italian natural rind Fontina PDO cheese	Italian natural rind Casera Valtellina PDO cheese	Italian Formaggio di Fossa cheese	Austrian washed rind Vorarlberger Bergkase cheese	Italian Gorgonzola PDO cheese	British Stilton Blue cheese	Belgian washed rind cheese : Herve	American mold rind cheeses	Frequency (%) ³	11 Irish washed rind cheese	137 european and american cheese rinds	Frequency (%) ⁴
	CD	CD	CD	CI	CI	CI	CI	CI	CI	HTS	HTS		HTS	HTS		
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3 id 36 Proteobacteria).

4 08); Valdès-Stauber *et al.* (1997); Larpin *et al.* (2006); Larpin-Laborde *et al.* (2011); Mounier *et al.*

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8 ification via molecular biology methods such as PCR-DGGE/TGGE, PCR-CE-SSCP, and 16S rDNA
9 ification via 16S rDNA amplicon sequencing (454 pyrosequencing or Illumina).

10 gley *et al.*, 2012 and Wolfe *et al.*, 2014.

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(2009); Mounier *et al.* (2005); Ogier *et al.* (2004); Feurer *et al.* (2004); Bleicher *et al.* (2010); Mac

cloning/sequencing.

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4 *oz et al. (2003); Parayre et al. (2007); Rea et al. (2007); Gori et al. (2013); Fontana et al. (2010); F*
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aligni *et al.* (2012); Bockulich *et al.* (2013); Amato *et al.* (2012); Roth *et al.* (2010); Dolci *et al.* (20

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4 09); Dolci *et al.* (2013); Schornsteiner *et al.* (2014); Ercolini *et al.* (2003); Quigley *et al.* (2012); Wc
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Olfe *et al.* (2014) and Delcenserie *et al.*, (2014).

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