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Staphylococcus aureus virulence and metabolism are dramatically affected by Lactococcus lactis in cheese matrix

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1 **_Staphylococcus aureus_ virulence and metabolism are dramatically affected**
2 **by _Lactococcus lactis_ in cheese matrix**

3

4 Running title: *S. aureus* interaction with *L. lactis* in cheese matrix.

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6 Marina Cretenet[1,2], Sébastien Nouaille[3,4,5], Jennifer Riviere[1,2], Lucie Gouffier[1,2], Ludwig
7 Stenz[6,7], Patrice François[6,7], Jacques-Antoine Hennekinne[8], Michel Piot[1,2], Marie Bernadette
8 Maillard[1,2], Jacques Fauquant[1,2], Pascal Loubière[3,4,5], Yves Le Loir[1,2] and Sergine Even[1,2]*

9

10 [1] INRA, UMR1253 STLO, 85 rue de Saint Brieuc, F-35000 Rennes, France.
11 [2] Agrocampus Ouest, UMR1253 STLO, 85 rue de Saint Brieuc, F-35000 Rennes, France
12 [3] Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077
13 Toulouse, France.
14 [4] INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse,
15 France.
16 [5] CNRS, UMR5504, F-31400 Toulouse, France.
17 [6] Genomic Research Laboratory, University of Geneva Hospitals, Rue Gabrielle-Perret-
18 Gentil, 4 CH-1211 Geneva 14 – Switzerland
19 [7] University Medical Center, Department of Microbiology and Molecular Medicine,
20 CH-1211 Geneva 4, Switzerland
21 [8] Laboratory for Study and Research on Quality of Food and on Food Processes, French
22 Agency for Food Safety (AFSSA-lerqap), National and Community Reference Laboratory,
23 94704 Maisons-Alfort cedex, France

24

25

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26 *corresponding author

27 Tel +33 (0) 2 23 48 59 44

28 Fax +33 (0) 2 23 48 53 50

29 sergine.even@rennes.inra.fr

30 Correspondence address:

31 Sergine Even, UMR1253 STLO INRA-Agrocampus Ouest, 85 rue de Saint Brieuc, F-35000

32 Rennes Cedex, France

33

34 **Keywords**: *Staphylococcus aureus*, cheese, virulence, enterotoxin, ecosystem, bacterial

35 interactions

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36 **Summary**

37 In complex environments such as cheeses, the lack of relevant information on the physiology
38 and virulence expression of pathogenic bacteria and the impact of endogenous microbiota has
39 hindered progress in risk assessment and control. Here, we investigated the behaviour of
40 *Staphylococcus aureus*, a major foodborne pathogen, in a cheese matrix, either alone or in the
41 presence of *Lactococcus lactis,* as a dominant species of cheese ecosystems. The dynamics of
42 *S. aureus* was explored *in situ* by coupling a microbiological and, for the first time, a
43 transcriptomic approach. *L. lactis* affected the carbohydrate and nitrogen metabolisms and the
44 stress response of *S. aureus* by acidifying, proteolysing and decreasing the redox potential of
45 the cheese matrix. Enterotoxin expression was positively or negatively modulated by both *L.*
46 *lactis* and the cheese matrix itself, depending on the enterotoxin type. Among the main
47 enterotoxins involved in staphylococcal food poisoning, *sea* expression was slightly favoured
48 in the presence of *L. Lactis*, whereas a strong repression of *sec4* was observed in cheese
49 matrix, even in the absence of *L. lactis*, and correlated with a reduced *saeRS* expression.
50 Remarkably, the *agr* system was downregulated by the presence of *L. lactis*, in part because
51 of the decrease in pH. This study highlights the intimate link between environment,
52 metabolism and virulence, as illustrated by the influence of the cheese matrix context,
53 including the presence of *L. lactis*, on two major virulence regulators, the *agr* system and
54 *saeRS*.

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58 **Introduction**

59 *Staphylococcus aureus* is a major human pathogen, with clinical manifestations ranging from
60 skin infections to sepsis (Wertheim et al., 2005). *S. aureus* is also a major causative agent of
61 food poisonings, due to the production of staphylococcal enterotoxins (SE) by strains
62 contaminating foodstuffs (Zhang et al., 1998; Tirado and Schmidt, 2001; Le Loir et al., 2003;
63 Ikeda et al., 2005). Although symptoms are not life-threatening or only rarely so,
64 staphylococcal food poisoning (SFP) outbreaks remain a persistent public health problem.
65 Milk and milk products are often incriminated in SFP, especially in France (De Buyser et al.,
66 2001; Jorgensen et al., 2005; Delbes et al., 2006).

67 The capacity of *S. aureus* to provoke one or another infection relies on a wide variety of
68 virulence factors, the expression of which reportedly depends on the nature of the
69 environment and stresses encountered. This intimate link between metabolic processes and
70 virulence gene expression in *S. aureus* has been recently highlighted by Somerville and
71 Proctor (Somerville and Proctor, 2009). In recent years, functional genomics, transcriptomics
72 and proteomics have led to significant results on virulence gene expression and regulation and
73 on the responses of *S. aureus* to different stressors (Dunman et al., 2001; Bischoff et al., 2004;
74 Weinrick et al., 2004; Cui et al., 2005; Chang et al., 2006; Pane-Farre et al., 2006; Rogasch et
75 al., 2006). However these studies were conducted either in laboratory conditions or in a
76 setting relevant to the medical context and thus are hardly transferable to complex food
77 environments.

78 *S. aureus* growth and enterotoxin detection have been reported for years in several foodstuffs
79 including milk products (Vernozy-Rozand et al., 1998; Meyrand et al., 1998; Delbes et al.,
80 2006; Poli et al., 2007; Cremonesi et al., 2007). More recently, a few studies have investigated
81 *S. aureus* behaviour in milk, allowing relevant genes in this context to be pointed out
82 (Lammers et al., 2000; Sharer et al., 2003; Borezee-Durant et al., 2009). Nevertheless, apart

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83 from these few recent and targeted studies, the physiology of *S. aureus* and the mechanisms
84 governing its virulence expression are poorly understood in the milk and cheese context. In
85 particular, our knowledge of individual enterotoxin gene expression and regulation was
86 mainly derived from studies performed in laboratory media and in planktonic growth
87 conditions (Derzelle et al., 2009). Recently, technical bottlenecks have been overcome,
88 allowing the measurement of gene expression, including expression of enterotoxin genes, in
89 complex matrices such as cheeses (Ulve et al., 2008; Duquenne et al., 2010). As a
90 consequence of these advances, *in situ* approaches can now be used to investigate *S. aureus*
91 physiology, including virulence expression, in realistic environmental conditions.
92 Several factors can affect *S. aureus* behaviour in cheese. In particular, the presence of other
93 microorganisms, especially lactic acid bacteria (LAB), reportedly limits *S. aureus* growth and
94 enterotoxin production. However, again, most of our knowledge relies on observations or
95 screenings for inhibitory activities, the molecular mechanisms of which remain unknown.
96 LAB antagonism is a complex and multifactorial phenomenon, and various hypotheses have
97 been advanced regarding its mechanisms, including production of inhibitory compounds
98 (bacteriocin, hydrogen peroxide), acidification, and nutritional competition (Barber and
99 Deibel, 1972; Haines and Harmon, 1973; Notermans and Heuvelman, 1983; Ammor et al.,
100 2006; Delbes et al., 2006; Otero and Nader-Macias, 2006; Charlier et al., 2008a). Molecular
101 and global approaches, including transcriptomics and proteomics, are powerful tools that have
102 already allowed the mechanisms of action of one species of bacteria upon another to be
103 deciphered (Mashburn et al., 2005; Laughton et al., 2006). Recently, we reported the first
104 transcriptomic approach regarding the interaction between *S. aureus* and *L. lactis* in a
105 chemically defined medium (CDM) (Even et al., 2009; Nouaille et al., 2009). We showed that
106 *L. lactis* was able to strongly affect the expression of several regulatory systems and virulence

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107 genes of *S. aureus*, including the accessory gene regulator (*agr),* a key regulator of bacterial
108 virulence.
109 In this study, *S. aureus* MW2 growth was followed in cheese matrix for 7 days by coupling a
110 microbiological and, for the first time, a transcriptomic approach. *S. aureus* was grown either
111 alone or in the presence of *L. Lactis* in order to address the influence of this antagonistic flora.
112 Results show that, in cheese matrix, expression of important *S. aureus* virulence genes was
113 strongly altered by both the cheese matrix itself, as an abiotic environmental component, and
114 by the presence of *L. lactis*.
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117 **Results**

118 **One log inhibition of *S. aureus* growth by *L. lactis* in cheese matrix.** *S. aureus* MW2 was
119 grown in ultra-filtrate (UF) retentate, a cheese matrix, as previously described (Ulve et al.,
120 2008) to mimic the conditions encountered during the cheese-making process (i.e.,
121 immobilized growth in the colony in a dairy environment). UF retentate, the cheese matrix
122 used for this study, is based on the MMV (Maubois-Moquot-Vassal) process and has been
123 widely used in several cheese-making processes for soft to semi-hard cheeses (Mistry V.V.
124 and Maubois J.L., 1993; Maubois J.L., 1997). MW2 was selected among all sequenced strains
125 of *S. aureus* for its origin (community-acquired) and its content in enterotoxin genes (Baba et
126 al., 2002). Two additional *S. aureus* field strains (kindly provided by AFSSA-lerqap) involved
127 in SFP outbreaks were tested in similar growth conditions to validate our results: *S. aureus*
128 257D (isolated from chocolate milk and carrying the *sea* gene) and *S. aureus* 253E (isolated
129 from cheese and carrying the *sea* and *sed* genes).
130 *S. aureus* MW2 grew exponentially in the cheese matrix for the first 6 h of culture and entered
131 into the stationary phase before the temperature shift at 10 h. The final population density was
132 2 x 10^9 cfu g^{-1} at 24 h and was maintained until day 7 (Fig. 1). The kinetics of *S. aureus* MW2
133 growth in mixed culture revealed an inhibition of *S. aureus* by *L. lactis* from 6 h onwards,
134 reaching one log at 7 days (Fig. 1A). Lactose was never depleted from the medium during the
135 7 days of culture (Fig. 1B). In *S. aureus* pure culture, small amounts of acetate were
136 quantified by day 7, whereas lactate production was not observed (Fig. 1B). pH remained
137 unchanged. In contrast, the presence of *L. lactis* caused a drop in pH values down to 5 by day
138 7, in accordance with lactate and acetate production (Fig. 1C). Similar inhibition by *L. lactis*
139 was observed on *S. aureus* 257D and 253E, the two field strains. Both strains grew to a final
140 density (10^9 cfu g^{-1} by day 7) in pure culture in cheese matrix that was similar to that of the *S.*

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141 *aureus* MW2 strain. Final populations of strains 257D and 253E after 7 days were 1.5- and 2-
142 log lower, respectively, in mixed culture compared to pure culture (data not shown).
143
144 **A severe decrease of the expression of genes involved in cellular machinery in the**
145 **presence of *L. lactis***. The gene expression profiles of *S. aureus* in pure and mixed cultures
146 with *L. lactis* in cheese matrix were analysed using microarrays and reverse transcription
147 quantitative PCR (RT-qPCR) over 7 days. Expression of genes involved in cell division,
148 transcription, translation, and cell envelope biosynthesis were maintained for 7 days in pure
149 culture but were downregulated in mixed cultures (Table 1, Table S1). Indeed, the expression
150 of genes involved in cell division (*ftsH*, *ftsZ, ftsL*) and cell envelope biosynthesis (*pbp4, gpsA,*
151 *MW2013)* continuously decreased during the first 24 h in mixed culture but was not
152 significantly modified in pure culture. Additionally, expression of genes involved in
153 transcription (*rho, rpoC*) and translation (*fus, tufA, rpmI*) decreased in mixed culture at all
154 times compared to 6 h. These results were confirmed by RT-qPCR and expanded to 7 days of
155 culture for *ftsZ* and *pbp4* (Table 1). In conclusion, although *S. aureus* entered a stationary
156 phase in pure culture after 24 h, cells retained an active metabolic status, as revealed by the
157 maintenance of expression of several genes related to growth, transcription and translation.
158 The presence of *L. lactis* provoked a premature arrest of *S. aureus* MW2 growth.
159 Accordingly, expression of growth-related genes decreased in mixed compared to pure
160 culture.
161
162 **A major acid stress triggered by *L. lactis***. *S. aureus* did not undergo major stresses when
163 grown in pure culture in cheese matrix (Table 1, Table S1). Indeed, no specific stress response
164 was revealed by the transcriptomic profile in pure culture except the induction of the SigmaB
165 regulon, in agreement with the transition to the stationary phase. Conversely, the presence of

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166 *L. lactis* altered the expression of stress-related genes. In agreement with cheese matrix
167 acidification by *L. lactis*, genes associated with the acid stress response showed increased
168 expression in mixed culture, as revealed by both microarray and RT-qPCR data. The urease
169 (*ureD*) pathway, as well as part of the deaminase pathway (*arcC, arcR*) involved in the acid
170 stress response (Cotter and Hill, 2003), were overexpressed in mixed compared to pure
171 culture (Table 1). Accordingly, urea was consumed more rapidly in mixed culture than in pure
172 culture (Table S2). Urea can indeed only be consumed by *S. aureus*, as *L. lactis* does not
173 possess a urease-encoding gene. The *sodA* gene, encoding a superoxide dismutase and
174 reportedly involved in the response to oxidative stress generated by an acidic environment
175 (Clements et al., 1999), was also highly upregulated in mixed culture, with a ~4–fold increase
176 at 24 h and 7 days compared to pure culture (Table 1). In addition, expression of members of
177 the CtsR regulon, including the chaperone-encoding genes *dnaK* and *clpC,* as well as *ctsR*
178 itself, increased in mixed culture over 7 days (Table 1), as previously reported in response to
179 various stresses, including acid stress (Chastanet et al., 2003).

180 Apart from the induction of acid stress–related gene expression, *S. aureus* did not undergo
181 major stresses in the presence of *L. lactis*. Genes associated with the general stress response
182 were underexpressed in mixed culture. Notably, genes known to be under SigmaB control (eg,
183 *asp23*, MW1682 and MW0781, encoding, respectively, the alkaline shock protein 23 and two
184 general stress protein-like proteins) were underexpressed in mixed compared to pure cultures
185 (Table S1, Table 1). The expression of *sigB* itself had decreased in mixed culture by day 7
186 (Table 1). SigmaB activity is regulated at the protein rather than the transcriptional level
187 (Senn et al., 2005), and SigmaB response to stressful conditions is reportedly primarily
188 controlled by an induction of *rsbU* transcription (Pane-Farre et al., 2009). Here, *rsbU* was also
189 downregulated in mixed culture (Table S1), which may account for the reduced activity of
190 SigmaB and thus the lower expression of the SigmaB regulon.

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191 Interestingly, genes known to be involved in the oxidative stress response (ie, *trxA*, encoding
192 the thioredoxin, and *katA*, encoding a catalase) were down-expressed in the mixed compared
193 to the pure culture (Table 1). Likewise, the expression of *ahpF*, encoding the alkyl
194 hydroperoxide reductase subunit F, was induced in pure culture whereas it remained constant
195 in mixed culture over 24 h (Table S1). Collectively, these data suggest that *S. aureus* cells
196 sensed a lower redox potential in mixed culture, in accordance with the reducing activity of *L.*
197 *lactis* (Tachon et al., 2010).
198 In conclusion, response to acid stress seemed predominant over any other stress-specific
199 response encountered by *S. aureus* during growth in mixed culture with *L. lactis*.
200
201 **Major changes in *S. aureus* carbohydrate metabolism**. In cheese matrix, lactose
202 concentration decreased only slightly by day 7 in pure culture, suggesting that *S. aureus* did
203 not actively metabolize lactose as a carbon source (Fig. 1B). *S. aureus* can grow on amino
204 acids, particularly glutamate (Mah et al., 1967; Borezee-Durant et al., 2009). Predictably, the
205 glutamate concentration strongly decreased until 24 h in pure culture (Table S2). Whether
206 glutamate was also used as a carbon source by *S. aureus* in mixed culture in cheese matrix can
207 be assumed but not proved, because in mixed culture changes in free amino acid and
208 carbohydrates resulted from both lactococcal and staphylococcal metabolisms. Indeed, several
209 amino acids, including glutamate, accumulated from 6 h onwards in cheese matrix in mixed
210 culture as a result of lactococcal proteolytic activity (Table S2). Glutamate was available in
211 mixed culture to sustain *S. aureus* growth.
212 Strikingly, the presence of *L. lactis* provoked a shift of the expression of several genes related
213 to pyruvate metabolism. The expression of *lctE* and *adh1*, encoding lactate dehydrogenase
214 and alcohol dehydrogenase, was transiently induced in pure culture, whereas it decreased
215 from 10 h onwards in mixed culture (Table 1). On the contrary, the expression of *pflA*,

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216 encoding the pyruvate formate lyase, was higher in mixed culture from 24 h onwards. In the
217 mixed culture, *alsS* and *butA*, encoding respectively the alpha acetolactate synthase and the
218 acetoin reductase, were strongly over-expressed, at all time points in the case of *alsS* (Table
219 1). Of note, production of acetoin was detected in mixed culture (data not shown), although
220 we cannot exclude, as previously mentioned for amino acids and carbohydrates, that it
221 resulted from both lactococcal and staphylococcal activities. The expression of *alsS* is under
222 the control of CidR, which has been shown to be induced by acetate and lactate, together with
223 the *cid* and *lrg* operons (Yang et al., 2006). In addition, we found here that *cidA* and, to a
224 lesser extent, *lrgA* were overexpressed in mixed culture (Table 1), which correlated well with
225 the production of lactate and acetate by *L. lactis* in mixed culture (Fig. 1B).
226
227 ***L. lactis* proteolysis relieved *S. aureus* amino acid limitation in cheese matrix.** Cheese
228 matrices are reportedly poor in free amino acids, so the main source of amino acids is
229 provided by proteolysis of caseins (Savijoki et al., 2006). Accordingly, a biochemical analysis
230 revealed that very few amino acids were initially present in the UF retentate (Table S2). Most
231 of them disappeared before 6 h in *S. aureus* pure culture, suggesting a free amino-acid
232 limitation in cheese matrix similar to that previously observed in milk (Borezee-Durant et al.,
233 2009). A few amino acids had accumulated in pure culture by day 7, yet at a low level,
234 suggesting a limited but active proteolysis of *S. aureus* MW2 on caseins. Urea was also used
235 as an alternative nitrogen source after 6 h (Table S2). Urease is known to be induced in the
236 case of nitrogen starvation in *Bacillus subtilis* and *Corynebacterium glutamicum* (Schmid et
237 al., 2000; Brandenburg et al., 2002; Beckers et al., 2004).
238 In contrast, in mixed culture free amino acids were released and were thus available for the
239 growth of both lactococcal and staphylococcal populations (Table S2). Amino acid
240 accumulation is likely to result mainly from lactococcal proteolytic activity. Amino acid

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241 release might thus relieve the limitation observed in *S. aureus* pure culture. Indeed, the
242 expression of *relA,* a gene involved in the stringent response, that is induced in the case of
243 amino acid limitation (Anderson et al., 2006), was significantly reduced in mixed culture
244 (ratios 0.2 at 24 h and 0.1 at day 7). Furthermore, several genes involved in nitrogen nutrition
245 and amino acid biosynthesis were downregulated during mixed culture. Genes encoding
246 peptidases such as *pepT* and MW1694 as well as *opp3*, the sole oligopeptide transport system
247 used for growth in milk (Hiron et al., 2007), were downregulated in mixed culture (Table 1,
248 Table S1). Expression of genes involved in the biosynthesis pathway of leucine (*leuA*) and
249 isoleucine (*ilvD*) was also lower in mixed culture, especially at 24 h (Table 1). Biosynthesis of
250 these branched-chain amino acids (BCAA: leucine, isoleucine and valine) is under the control
251 of the CodY repressor (Pohl et al., 2009). Increased proteolytic activity in the mixed culture
252 likely provided the necessary co-repressors (BCAA) for CodY-mediated repression of
253 branched-chain amino acid biosynthesis. Collectively, these results suggest that, in mixed
254 culture, lactococcal proteolysis deeply modified the expression of *S. aureus* genes involved in
255 nitrogen nutrition and amino acid biosynthesis.
256
257 **Downregulation of the expression of *S. aureus agr* system in mixed culture**. *S. aureus*
258 virulence expression is tightly controlled by several regulatory systems, of which the *agr*
259 system, *sarA*, *saeRS* and *codY* are key elements (Novick, 2003; Bronner et al., 2004). A
260 striking difference was noted in *agr* system expression when pure and mixed cultures were
261 compared. In pure culture, both *agrA* and RNAIII (*agr* effector) expressions increased over
262 time, especially at 24 h and 7 days, whereas these inductions were strongly attenuated in the
263 presence of *L. lactis* (Table 1). Hence, in mixed culture, the expression of *agrA* and RNAIII
264 increased slightly at 10 h but decreased afterward. As a result, the expression of RNAIII and
265 *agrA* was strongly downregulated from 24h onward in mixed culture with *L. lactis* compared

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266 to pure culture. Accordingly, expression of *rot*, which is normally repressed by RNAIII in the
267 stationary phase (Geisinger et al., 2006), was higher in mixed culture at 7 days (Table 1).
268 Likewise, expression of *sarA* and *mgrA,* both known as activators of the *agr* system (Chien et
269 al., 1998; Ingavale et al., 2005), were downregulated at 7 days in mixed compared to pure
270 cultures (Table 1). The downregulation of the *agr* system by *L. lactis* was confirmed in the
271 field strain *S. aureus* 257D: the expression of both *agrA* and RNAIII was strongly enhanced
272 in pure culture in cheese matrix and severely diminished in mixed culture (data not shown).
273 No transcripts of *agrA* and RNAIII were quantified in *S. aureus* 253E, indicating that this
274 strain was *agr* deficient.
275 Regarding virulence factors, differential expression of several genes occurred in the presence
276 of *L. lactis*. Some cell wall–associated virulence factors showed enhanced expression over 24
277 h in *S. aureus* pure cultures only (e.g. *fnb* (MW0764) and *ebpS*, encoding fibrinogen and
278 elastin-binding proteins, respectively; Table S1). Other genes were induced in both conditions
279 but earlier in pure culture, as observed for *clfA* (clumping factor A) and *cap8A* (involved in
280 the capsular polysaccharide synthesis), compared to mixed culture (Table 1). Additionally,
281 *ssaA* (staphylococcus secretory antigen SsaA homolog) was over-expressed in pure culture
282 from 6 h to 24 h (Table S1). Conversely, *fnb* (MW2421), *sdrD* and *sdrC* encoding fibrinogen-
283 binding proteins were over-expressed in mixed culture (Table 1, Table S1). Finally, the
284 biofilm-associated gene *icaA* was strongly downregulated in mixed compared to pure culture
285 (Table 1). Few secreted virulence factors showed differential expression in pure and mixed
286 cultures of *S. aureus*. The hemolysin III (MW2096)– and hemolysin (MW0802)–encoding
287 genes showed increased expression in pure culture from 6 h to 24 h, while their expression
288 remained constant in mixed culture during this period (Table S1). Conversely, *isaB*
289 (immunodominant antigen B) expression was induced earlier in pure culture but reached a
290 higher level in the mixed culture than in the pure culture at 7 days (14.3-fold) (Table 1).

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291 In conclusion, few virulence genes showed differential expression between pure and mixed
292 cultures, and no general trends were observed. Strikingly, downregulation of the *agr* system
293 was provoked by *L. lactis*, as previously observed in a chemically defined medium under
294 environmental conditions very different from those of the cheese matrix (Even et al., 2009).
295
296 **CodY-independent inhibition of *S. aureus* virulence by *L. lactis*.** Transcriptomic analysis
297 of *S. aureus* MW2 suggested an increased CodY repression in mixed culture with *L. lactis.*
298 Increased proteolytic activity in mixed culture was indeed likely to relieve the amino acid
299 limitation that occurred in pure culture and to provide the co-repressors (valine, isoleucine
300 and leucine) for CodY-mediated repression. Recently, it was shown that a mutation in *codY*
301 was associated with an increased expression of both RNAII and RNAIII (Majerczyk et al.,
302 2008). We thus tested whether the higher CodY activity in mixed culture resulting from *L.*
303 *lactis* proteolysis could be responsible for the downregulation of the *agr* system. To address
304 this question, a *S. aureus* MW2 *ΔcodY* mutant strain was constructed and tested in pure and
305 mixed cultures. In pure culture, a higher expression of RNAIII expression was observed in the
306 *codY* mutant as previously described by Majerczyk et al. The downregulation of both *agrA*
307 and RNAIII by *L. lactis* occurred in the *codY* mutant as in the wild-type strain (Fig. 2). These
308 results indicated that the downregulation of the *S. aureus agr* system by *L. lactis* was not
309 mediated through CodY repression and thus was probably not related to the proteolytic
310 activity of *L. lactis;* however, a CodY-independent regulation pathway could not be excluded.
311
312 **Acidification by *L. lactis* is partially involved in *S. aureus* virulence inhibition**. To further
313 evaluate the mechanisms involved in inhibition of *S. aureus* growth and lack of induction of
314 the *agr* system expression in mixed culture, the decrease in pH was dissociated from the
315 presence of *L. lactis*. Glucono delta-lactone (GDL) was used to mimic acidification by *L.*

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316 *lactis*. Compared to other acids, spontaneous GDL hydrolysis into gluconic acid is slow,
317 occurs in the cheese matrix after curd formation and mimics the kinetics of the decrease in pH
318 induced by *L. lactis* (Fig. 3B). *S. aureus* growth was not significantly affected by the addition
319 of GDL (Fig. 3A). We investigated the effect of acidification on the expression of the *agr*
320 system in the three environments and found dissimilar effects on the transcription of RNAII
321 and RNAIII. Expression of *agrA* was decreased only in mixed culture, whereas RNAIII
322 expression was reduced by both GDL acidification and mixed culture conditions (Fig. 3C and
323 3D). However, it must be noted that RNAIII reduction was only 4-fold in GDL conditions
324 compared to 80-fold in mixed culture. These results demonstrate that, in cheese matrix, the
325 decrease in pH in mixed culture could partially account for RNAIII downregulation. Finally,
326 it should be noted that acidification directly inhibited the P3 promoter (RNAIII)
327 independently of an effect on the autoinducing system (RNAII).
328
329 **Downregulation of enterotoxin gene expression in cheese matrix.** We especially focused
330 on the production of enterotoxins because they are essential causative agents of SFP. Of the
331 six enterotoxins possessed by *S. aureus* MW2, SEA, SEC, SEG and SEH have a demonstrated
332 emetic activity, whereas SElL and SElK are non-emetic (Orwin et al., 2001; Thomas et al.,
333 2007). The expression profiles of enterotoxin genes were heterogeneous with respect to time
334 and *L. lactis* effect. In *S. aureus* pure culture in cheese matrix, *sea* and *seh* expression
335 decreased with time (6-fold and 8-fold, respectively, between 6 h and 7 days; Table 1). In
336 contrast, *sec4* expression did not vary significantly over 7 days, and expression of *seg2, sek2*
337 and *sel2* decreased only slightly with time. While *seg2* and *sek2* were under-expressed in
338 mixed culture from 10 h onwards, no significant variation was observed for *sec4* expression in
339 the mixed *vs* the pure culture. On the contrary, both *sel2* and *seh* genes were over-expressed
340 in the mixed compared to the pure culture, with a maximum ratio of 17-fold for *seh* at 7 days

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341 (Table 1). The expression profile of *sea* was similar in mixed and pure cultures, and *sea*
342 expression showed a slight increase in mixed culture. Results obtained with *S. aureus* field
343 strains isolated from SFP confirmed that *sea* expression was not affected by the presence of *L.*
344 *lactis* LD61 and even significantly increased in *S. aureus* 257D. Indeed, *sea* expression
345 decreased with time in both pure and mixed cultures in the field strain *S. aureus* 253E,
346 without any significant effect of the presence of *L. lactis*. An inductive effect of *L. lactis* on
347 *sea* expression was observed in the field strain 257D: *sea* expression was higher in mixed
348 compared to pure culture at both 9 h and 7 days (data not shown).
349 The kinetics of enterotoxin expression in pure *S. aureus* culture in cheese matrix were in
350 agreement with previously published data obtained in BHI, a rich laboratory medium, for *sea,*
351 *seg2* and *sek* (Derzelle et al., 2009). Under planktonic growth conditions in BHI, expression
352 of *sec, seh* and *sel* was enhanced after the exponential growth phase (Derzelle et al., 2009),
353 whereas in the cheese matrix expression of these genes did not change or was decreased
354 (Table 1). Enterotoxin expression was further compared in *S. aureus* MW2 between growth in
355 pure culture in cheese matrix and in chemically defined medium (CDM) under planktonic
356 conditions over 24 h, corresponding to the late stationary phase (Table 2, (Even et al., 2009)).
357 The influence of the cheese matrix clearly varied with the enterotoxin types. The expression
358 profile of *sea* was similar in both conditions, and *seg2* and *sek2* expressions were slightly
359 lower in cheese matrix compared to CDM during the exponential growth phase (2- to 3-fold at
360 8 h). Strikingly, the cheese matrix strongly altered both the level and dynamics of *sec4, seh*
361 and *sel2* expression. *sec4, seh* and *sel2* expressions were higher in CDM compared to cheese
362 matrix at all time-points, from 3- to 23-fold for *sec4*, 5- to 64-fold for *seh*, and from 4- to 42-
363 fold for *sel2* (Table 2). Although *seh* expression remained constant in CDM over 24 h (Even
364 et al., 2009), it strongly decreased in the stationary phase (24 h) in cheese matrix (Table 1). In
365 addition, *sec4* and *sel2* expression strongly increased in CDM in the stationary phase (Even et

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366 al., 2009) but remained constant in cheese matrix and even slightly decreased at 24 h (Table
367 1). The differences in *sec4* expression in cheese matrix versus CDM were validated by
368 immunological detection of SEC production. Production of 5.16 µg ml^{-1} of SEC by *S. aureus*
369 MW2 was detected after 24 h in CDM, wherease no SEC was detected at 24 h in cheese
370 matrix. In cheese matrix, SEC was detectable but not quantifiable (quantification limit: 0.021
371 ng ml^{-1}) at 7 days.
372 In conclusion, these results show that both the cheese matrix itself and the presence of *L.*
373 *lactis* modulated enterotoxin gene expression and that these modulations were strongly
374 dependent on enterotoxin type.
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379 **Discussion**

380 *S. aureus* physiology, including virulence expression and regulation, has been widely studied
381 in the past decades by either targeted or global strategies. However, most of them have been
382 done in laboratory growth conditions or in vivo conditions relevant to human or animal
383 infectious contexts and far removed from the context of food such as cheese matrix. *S. aureus*
384 growth conditions in cheese matrix differ from classical laboratory conditions in at least 2
385 major ways. Milk is a complex nutritional matrix that undergoes several physico-chemical
386 modifications, in a concomitant or sequential way, including transition from liquid to solid
387 state. As a consequence, *S. aureus* growth in liquid medium is planktonic, whereas in cheese
388 matrix *S. aureus* is immobilised and grows as colonies. In addition, cheeses contain more or
389 less complex microbial ecosystems, including acidifying flora such as LAB that have been
390 shown to display antagonistic properties against *S. aureus*. In this study, we investigated for
391 the first time the dynamics of *S. aureus* growth and global gene expression in a cheese matrix
392 for 7 days, allowing a global understanding of *S. aureus* physiology in this particular context,
393 with special attention paid to the influence of *L. lactis*, the model LAB species.

394

395 **Premature arrest of *S. aureus* growth in the presence of *L. lactis*.** The overall view of the
396 *S. aureus* transcriptome in mixed culture with *L. lactis* showed a massive downregulation of
397 genes involved in cell division, transcription, translation and cell envelope metabolism in
398 comparison to the pure culture. Growth of *S. aureus* stopped earlier in mixed culture,
399 resulting in one log inhibition at 24 h. Then, between 24 h and 7 days, the population
400 remained viable. *S. aureus* growth slowed earlier (at 6 h) in mixed culture and at a non-
401 limiting pH (6.3) since *S. aureus* can grow in a more acidic environment (Charlier et al.,
402 2008b). Hence, acidification did not seem to be involved in the early inhibition of *S. aureus*
403 growth. This was further reinforced by GDL experiments, which revealed that the decrease in

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404 pH did not affect *S. aureus* growth during the course of the 7-day study. However, it must be
405 noted that the acid used to mimic acidification was not lactate and that the nature of the acid
406 used to adjust pH in the culture medium reportedly influences the impact of pH on *S. aureus*
407 growth (Domenech et al., 1992). A previously reported early inhibition of *S. aureus* growth
408 by *L. lactis* in liquid milk was not due to acidification but rather to unidentified nutrient-
409 related phenomena (Charlier et al., 2008b). The early inhibition of *S. aureus* growth observed
410 in the cheese matrix likely relied on similar mechanisms.
411
412 **Major acid stress but no general stress.** *S. aureus* did not undergo major stresses when
413 cultivated alone in cheese matrix, whereas the acid stress response was major in the *S. aureus*
414 transcriptomic profile in mixed culture, with induction of the expression of genes encoding
415 detoxification enzymes (*ureD, sodA*), the arginine deaminase pathway (*arcC, arcR*) and the
416 *ctsR* regulon (*ctsR, dnaK, clpC*).
417 *S. aureus* sensed not only the decrease in pH but also the production of lactate (and acetate)
418 itself that triggered major changes in central metabolism. Acidification of the cheese matrix
419 by *L. lactis* led to transcription of *S. aureus* genes involved in acetoïn production (*alsS* and
420 *butA*) instead of the gene encoding lactate dehydrogenase. The induction of *alsS* expression in
421 the presence of lactate or acetate is mediated by CidR (Yang et al., 2006). Concomitantly,
422 lactate and acetate induced the expression of *cid* and *lrg* operons in mixed culture. These two
423 operons were shown to affect *S. aureus* murein hydrolase activity, autolysis, viability and
424 biofilm formation (Rice et al., 2003; Rice et al., 2005; Rice et al., 2007). In particular, lactate
425 was shown to promote a transition of cell physiology towards a “survival” state, in which
426 cells remained viable with a reduced metabolic activity. Apart from acidic stress, no other
427 major stress was revealed in the *S. aureus* transcriptome in mixed culture.

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428 It should be noted that the presence of *L. lactis* relieved the amino acid limitation of *S. aureus*
429 in cheese matrix. The high proteolytic activity of *L. lactis* in cheese matrix resulted in the
430 release of free amino acids (especially branched-chain amino acids, co-factors of CodY) that
431 were then available for *S. aureus*. In mixed culture, we observed a strong repression of
432 leucine and isoleucine biosynthesis pathways, suggesting CodY-mediated activity (Pohl et al.,
433 2009).

434

435 **S. *aureus* sensed a lower redox potential in mixed culture.** Expression of genes belonging
436 to various functional categories (stress, carbon metabolism and virulence) suggested the redox
437 potential was lower in mixed culture. First, genes involved in oxidative stress response,
438 including *trxA, katA,* and *ahpF,* were upregulated in pure compared to mixed culture. In
439 addition, the expression of *clpL*, known to be under SigmaB control, was more than 7-fold
440 higher in mixed culture than pure culture, whereas the expression of other SigmaB-dependent
441 genes was reduced. The expression of *clpL* has been shown to be strongly induced in an
442 anaerobic environment independently of any SigmaB induction (Fuchs et al., 2007). In our
443 study, *lacG* (lactose transporter), *pflA* (involved in acetate formation) as well as *isaB*
444 (immunodominant antigen B) were over-expressed in mixed compared to pure cultures. *lacA*,
445 another gene belonging to the *lac* operon, *pflA* and *isaB* were also found to be induced in
446 anaerobic conditions by Fuchs et al. Collectively, these results suggest that *S. aureus* cells
447 sensed a lower redox potential in mixed compared to pure cultures, as might be expected
448 given that the early steps of *L. lactis* growth reportedly result in oxygen exhaustion and
449 reduction of the growth medium (Brasca et al., 2007; Tachon et al., 2010).

450

451 **A biofilm-like behaviour in mixed culture?** In mixed culture, *S. aureus* responded to a more
452 acidic and anaerobic-like (lower redox potential) environment than in pure culture. These two

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453 environmental parameters are often associated with biofilm formation. Many of the genes
454 whose expression is induced in cheese matrix in mixed culture are associated with biofilm
455 formation in the literature. Comparison of *S. aureus* transcriptomes in biofilm *versus*
456 planktonic growth showed higher transcription of genes involved in the deiminase pathway
457 (*arc* genes), genes coding for urease (*ureA-G*), and the pyruvate formate lyase (*plflA*) as well
458 as a decreased expression of genes involved in nitrogen metabolism (*opp3, ilvE*) (Beenken et
459 al., 2004; Resch et al., 2005). Enhanced expression of *isaB* can be related to anaerobic
460 conditions (Fuchs et al., 2007) and enhanced expression of *sdrC* to mild acidic conditions
461 (Weinrick et al., 2004). Both expressions were shown to be induced in biofilm compared to
462 planktonic conditions (Resch et al., 2005). Of note, *cidA* was shown to contribute to biofilm
463 adherence by affecting cell lysis and the release of genomic DNA. Extracellular DNA has
464 been found in the biofilm matrix produced by *S. aureus* (Rice et al., 2007; Mann et al., 2009).
465 All these genes associated with *S. aureus* biofilm formation were upregulated in mixed
466 compared to pure cultures. Nevertheless, it should be noted that this biofilm-like behaviour of
467 *S. aureus* in mixed culture was *ica*-independent as *icaA* expression was strongly
468 downregulated in mixed culture. This strong downregulation of *icaA* expression in mixed
469 culture may indeed be due to higher repression by CodY (Majerczyk et al., 2008).
470 Collectively, our data suggest that the acidic and anaerobic environment generated by *L. lactis*
471 triggered the same metabolic pathways involved in biofilm formation.

472

473 **Inhibition of the expression of the *S. aureus agr* system**. In mixed culture with *L. lactis*, a
474 lack of induction of the *agr* system (both RNAII (*agrA*) and RNAIII) was observed in the
475 post-exponential growth phase. Several mechanisms and signalling pathways have been
476 shown to influence the expression of the *agr* system. Notably, growth in an acid environment
477 has been reported to affect *S. aureus* RNAIII expression; however, this remains a matter of

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478 debate (Regassa et al., 1992; Weinrick et al., 2004; Bore et al., 2007). Here, using GDL, we
479 demonstrated that, in the cheese matrix context, a decrease in pH could, at least in part,
480 account for RNAIII downregulation. The expression of *agrA* was not affected by the addition
481 of GDL, showing that the decrease in pH affected RNAIII level directly and was not
482 dependent for this effect on the autoinducing system (*agrABCD).* Such uncoupling between
483 RNAII and RNAIII expression has been previously reported (Xiong et al., 2002; Garzoni et
484 al., 2007). Interestingly, downregulation of the *agr* system (*agrA* and RNAIII) by *L. lactis* had
485 been previously observed in a chemically defined medium under environmental conditions far
486 removed from the cheese matrix context (Even et al., 2009). The mechanism(s) involved in
487 *agr* system attenuation in CDM were not elucidated, but it was established that they are not
488 related to acidification as pH was maintained at 6.6. Hence, although the ability of *L. lactis* to
489 alter the *agr* system expression was not restricted to specific conditions, this ability appears to
490 rely on multiple mechanisms acting on RNAII and or RNAIII.
491 In cheese matrix, one could hypothesize that lactococcal proteolysis acted on *agr* system
492 expression through a CodY-mediated regulation in *S. aureus*. However, although CodY
493 reportedly affects *agr* system expression (Majerczyk et al., 2008), it did not seem to be
494 involved here since a *codY* mutant of *S. aureus* MW2 and the wild-type strain had similar
495 expression profiles of the *agr* system.
496
497 **What does enterotoxin expression in cheese matrix imply for food poisoning?** In
498 foodstuff, SE production is the main risk associated with *S. aureus.* Strikingly, three
499 enterotoxins of *S. aureus*, namely *sec4, seh* and *sel2,* were strongly downregulated in cheese
500 matrix compared to a chemically defined medium. This observation at the gene expression
501 level was confirmed at the protein level by quantification of SEC. Our results on *sec4*
502 expression and production were in agreement with those of previous studies, which failed to

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503 detect SEC production in cheese by strains carrying *sec* genes and producing SEC in
504 laboratory media (Otero et al., 1990; Otero et al., 1993; Poli et al., 2007). In contrast, *sea*
505 expression was not affected in cheese matrix and the production of SEA was not inhibited,
506 perhaps accounting for its high frequency in SFP (Mossel and Van Netten, 1990; Lehn et al.,
507 1995; Omoe et al., 2005).

508 Regulation of SE genes is poorly understood and mainly restricted to classical SE such as
509 SEA and SEC. Expression of the *sec4* gene is reported to be under a positive control by the
510 *agr* system and SaeRS and to decrease in glucose-containing medium (independently of
511 medium acidification) and high salt concentration (Genigeorgis et al., 1971; Regassa et al.,
512 1992; Regassa and Betley, 1993; Voyich et al., 2009). The promoter of *saeRS* was shown to
513 be repressed by a high salt concentration, implying that water activity was also reduced
514 (Geiger et al., 2008). Taken together, these results establish a link between water activity and
515 expression of *sec4* via SaeRS regulation. In cheeses, water activity is lower than in a liquid
516 medium such as CDM (typical water activities of cheeses vary from 0.9 to 0.99) (Marcos,
517 1993). Here, water activity was estimated at 0.96 in UF retentate cheese matrix. In agreement,
518 *saeS* expression was found to be lower in cheese matrix than in CDM from 8 h onward (Table
519 2). Reduced water activity in the cheese matrix may thus partially account for the
520 downregulation of *saeRS,* which may in turn be responsible for low *sec4* expression.

521 In addition to the effect of the cheese matrix itself on SE expression, the presence of *L. lactis*
522 was able to alter SE production. Of note, the effect of *L. lactis* on SE expression differed
523 between CDM and cheese matrix, highlighting again the crucial role of environmental
524 conditions (Even et al., 2009). Among the main enterotoxins involved in SFP, *sea* expression
525 was slightly increased in cheese matrix in the *S. aureus* MW2 strain as well as in *S. aureus*
526 257D, by the presence of *L. lactis*. This higher *sea* expression in the presence of *L. lactis*
527 might be due to acidification, as was recently reported (Wallin-Carlquist et al., 2010). In this

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528 study, addition of acetic acid provoked an increase of *sea* expression. This was related to the
529 induction of the *sea*-encoding prophage and thus to an increase of intracellular *sea* gene copy
530 number. The genetic context of enterotoxin genes is thus likely to play a major role in the
531 control of enterotoxin gene expression. Enterotoxin genes are carried by different genetic
532 determinants: *sea, seg2* and *sek2* are carried by the same prophage, *sel2* and *sec4* are located
533 on the same genomic island and *seh* exists beyond the right boundary of the chromosome
534 cassette SCC*mec*, carrying the methicillin resistance gene (Baba et al., 2002). The expression
535 of SE genes might thus respond to various regulation mechanisms depending on their genetic
536 support. If we are to better understand the variations observed here for SE expression, further
537 studies will be required to unravel the impact of mixed culture conditions on *S. aureus* phage
538 and mobile genetic element biology.
539
540 **Conclusions**.
541 The behaviour and dynamic response of *S. aureus* to *L. lactis* antagonism was explored
542 through *in situ* gene-expression profiling in conditions mimicking the cheese-making process.
543 This study provides new insights into how *S. aureus* adapts to a changing environment and
544 during microbial competition. It also highlights the important role of environmental
545 conditions, thus reinforcing the requirement for *in situ* approaches. In particular, this study
546 showed that SFP occurrence in the cheese ecosystem is a multilayer problem involving both
547 *S. aureus* metabolism and virulence expression in response to environmental changes driven
548 by technological processes (temperature, renneting) and microbial activity (*L. lactis* growth
549 and impact on the cheese matrix). Such parameters, individually or in combination, affect *S.*
550 *aureus* behaviour through various regulatory pathways. Deciphering the molecular
551 mechanisms underlying *S. aureus* response(s) to these multifactorial and dynamic

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552 environmental changes in cheese matrix opens new avenues for the development of novel
553 prevention strategies against this major foodborne pathogen.
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556 **Experimental procedures**

557 **Bacterial strain and growth conditions.** *L. lactis* subsp. *lactis* biovar *diacetylactis* LD61
558 (kindly provided by R. Perrin, Soredab, La Boissière Ecole, France) and *S. aureus* MW2 were
559 used throughout this work. Two additional *S. aureus* strains isolated from foodstuffs involved
560 in food poisoning and kindly provided by AFSSA-lerqap were used in complementary
561 interaction experiments: *S. aureus* 257D (isolated from chocolate milk involved in SFP,
562 contains *sea*), and 253E (isolated from cheese involved in SFP, contains *sea, sed*). *S. aureus*
563 was grown in UF–cheeses in pure and mixed cultures with *L. lactis* LD61 as previously
564 described (Ulve et al., 2008). Briefly, the UF retentate was heated to 30 °C and inoculated
565 with *S. aureus* from subculture on Tryptic Soy Broth (TSB) at 10^6 cfu g^{-1} for assessment of
566 the worst-case scenario in case of contamination. When added, *L. lactis* LD61 was inoculated
567 at 10^6 cfu g^{-1} from subculture on M17. Rennet (Maxiren 180; DSM Food Specialities, Delft,
568 The Netherlands) was added simultaneously at a concentration of 0.3 µl ml^{-1}. After incubation
569 for 10 h at 30 °C, UF cheeses (20 g) were transferred to 12 °C to mimic the temperature shift
570 encountered by *S. aureus* strains during the making of soft cheese. *S. aureus* and *L. lactis*
571 populations were determined as previously described (Nouaille et al., 2009).

572 In order to investigate the effect of acidification on *S. aureus*, glucono-delta-lactone (GDL,
573 Hansen, Arpagon, France) was added sequentially to the cheese matrix after 5 h and 10 h of
574 growth to mimic the pH decrease in mixed culture.

575 **Chemical analysis of UF cheeses**. Determination of sugars, organic acids and free amino-
576 acid content was performed as described previously (Cretenet et al., submitted).

577 **Quantification of enterotoxin C production.** Detection and quantification of enterotoxin C
578 were performed by the EU-CRL screening method (Anonymous, 2007) and confirmed by the
579 EU-CRL confirmatory method when positive (Hennekinne et al., 2007).

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580 **Construction of the *codY* mutant strain.** A *codY* deletion mutant of *S. aureus* MW2 was
581 obtained by double crossing over as previously described by Hiron et al (Hiron et al., 2007),
582 using plasmid pCG29 constructed and described by Pohl et al (Pohl et al., 2009).
583 **RNA extraction and transcriptome analysis.** RNA samples were extracted from both pure
584 and mixed cultures at different time points (6 h, 8 h, 10 h, 24 h and 7 days) in order to monitor
585 the dynamic response of *S. aureus* MW2 in cheese matrix, with and without *L. lactis*. Briefly,
586 cells were separated from the cheese matrix as previously described (Ulve et al., 2008). RNA
587 samples from *S. aureus* in pure or mixed cultures were then obtained and quantified, and their
588 qualities were evaluated as described in Even et al (Even et al., 2009). Labelling and
589 hybridization using a dedicated microarray were performed as previously described (Even et
590 al., 2009). The expression profiles of *S. aureus* in pure and mixed cultures were analysed with
591 the R software (R Development Core Team, 2007) following the protocol from Even et al.
592 (Even et al., 2009). Hybridizations were carried out with a constant amount of total RNAs to
593 maintain the efficiency and reproducibility of the labelling and hybridisation steps. In mixed
594 cultures, RNA samples were characterized by a mixture of both lactococcal and
595 staphylococcal cDNA, resulting in lower signals. Total RNA yielded a signal (signal /noise
596 ratio >2) in 90% of the spots retained for statistical analysis in pure cultures, but this figure
597 decreased to 18% at 7 days in mixed culture. The sampling time 7 days was therefore
598 removed from our statistical analysis for insufficient quality. Statistical analyses were
599 performed in pure and mixed cultures only until 24 h of culture. Statistical analysis was
600 performed using the ANOVA test considering a P value and FDR lower than 0.05. Genes
601 showing at least a 2-fold change were considered to be differentially expressed. The
602 microarray data for *S. aureus* in pure and mixed cultures were deposited in the public
603 repository database ArrayExpress under the accession numbers E-MEXP-2617 and XXXX.
604 All genes showing statistically significant variations of expression with time with a minimum

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605 2-fold change in pure or mixed cultures are presented in Table S1. This first analysis was used
606 to reveal trends in gene expression and to establish a list of genes, the expression of which
607 was verified and completed by quantitative RT-qPCR over the complete duration of the
608 experiment (including day 7).

609 **Reverse transcription-quantitative PCR.** Reverse transcription-quantitative PCR (RT-
610 qPCR) were performed using the oligonucleotides listed in Table S3 as previously described
611 (Even et al., 2009), with three independent biological replicates for all experimental time
612 points. *gyrB*, was used as an internal standard for normalization as previously described
613 (Beenken et al., 2004; Bore et al., 2007). *gyrB* was not differentially expressed over time in
614 the microarray data in either the pure or the mixed culture. Gene expression was thus reported
615 relative to *gyrB* and calculated using the following formula: (copies of each gene/copies of
616 *gyrB*) X 10 in each cDNA preparation. Statistical analysis was performed using the ANOVA
617 test considering a P value lower than 0.05 to identify genes showing significant change in
618 expression with time. Similarly, statistical analysis was performed using the T-test
619 considering a P value lower than 0.05 to identify, for each time point, genes showing a
620 significant change in expression in pure *versus* mixed cultures.

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630 **References**

631 Reference List

632

633 Ammor,S., Tauveron,G., Dufour,E., and Chevallier,I. (2006) Antibacterial activity of lactic
634 acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale
635 facility: 2--Behaviour of pathogenic and spoilage bacteria in dual species biofilms including a
636 bacteriocin-like-producing lactic acid bacteria. *Food Control* **17:** 462-468.

637 Anderson,K.L., Roberts,C., Disz,T., Vonstein,V., Hwang,K., Overbeek,R. et al. (2006)
638 Characterization of the Staphylococcus aureus heat shock, cold shock, stringent, and SOS
639 responses and their effects on log-phase mRNA turnover. *J Bacteriol* **188:** 6739-6756.

640 Anonymous (2007) Commission regulation n°1441/2007 of 5 December 2007. *OJEU* **L322:**
641 12-29.

642 Baba,T., Takeuchi,F., Kuroda,M., Yuzawa,H., Aoki,K., Oguchi,A. et al. (2002) Genome and
643 virulence determinants of high virulence community-acquired MRSA. *Lancet* **359:** 1819-
644 1827.

645 Barber,L.E. and Deibel,R.H. (1972) Effect of pH and oxygen tension on staphylococcal
646 growth and enterotoxin formation in fermented sausage. *Appl Microbiol* **24:** 891-898.

647 Beckers,G., Bendt,A.K., Kramer,R., and Burkovski,A. (2004) Molecular identification of the
648 urea uptake system and transcriptional analysis of urea transporter- and urease-encoding
649 genes in Corynebacterium glutamicum. *Journal of Bacteriology* **186:** 7645-7652.

650 Beenken,K.E., Dunman,P.M., McAleese,F., Macapagal,D., Murphy,E., Projan,S.J. et al.
651 (2004) Global gene expression in Staphylococcus aureus biofilms. *J Bacteriol* **186:** 4665-
652 4684.

For Peer Review Only

653 Bischoff,M., Dunman,P., Kormanec,J., Macapagal,D., Murphy,E., Mounts,W. et al. (2004)
654 Microarray-based analysis of the Staphylococcus aureus sigmaB regulon. *J Bacteriol* **186:**
655 4085-4099.

656 Bore,E., Langsrud,S., Langsrud,O., Rode,T.M., and Holck,A. (2007) Acid-shock responses in
657 Staphylococcus aureus investigated by global gene expression analysis. *Microbiology* **153:**
658 2289-2303.

659 Borezee-Durant,E., Hiron,A., Piard,J.C., and Juillard,V. (2009) Dual Role of the Oligopeptide
660 Permease Opp3 during Growth of Staphylococcus aureus in Milk. *Applied and Environmental*
661 *Microbiology* **75:** 3355-3357.

662 Brandenburg,J.L., Wray,L.V., Beier,L., Jarmer,H., Saxild,H.H., and Fisher,S.H. (2002) Roles
663 of PucR, G1nR, and TnrA in regulating expression of the Bacillus subtilis ure p3 promoter.
664 *Journal of Bacteriology* **184:** 6060-6064.

665 Brasca,M., Morandi,S., Lodi,R., and Tamburini,A. (2007) Redox potential to discriminate
666 among species of lactic acid bacteria. *J Appl Microbiol* **103:** 1516-1524.

667 Bronner,S., Monteil,H., and Prevost,G. (2004) Regulation of virulence determinants in
668 Staphylococcus aureus: complexity and applications. *FEMS Microbiol Rev* **28:** 183-200.

669 Chang,W., Small,D.A., Toghrol,F., and Bentley,W.E. (2006) Global Transcriptome Analysis
670 of Staphylococcus aureus Response to Hydrogen Peroxide. *J Bacteriol* **188:** 1648-1659.

671 Charlier,C., Cretenet,M., Even,S., and Le Loir,Y. (2008a) Interactions between
672 *Staphylococcus aureus* and Lactic Acid Bacteria: an old story with new perspectives. *Int J*
673 *Food Microbiol* **in press**.

For Peer Review Only

674 Charlier,C., Even,S., Gautier,M., and Le Loir,Y. (2008b) Acidification is not involved in the
675 early inhibition of Staphylococcus aureus growth by Lactococcus lactis in milk. *International*
676 *Dairy Journal* **18:** 197-203.

677 Chastanet,A., Fert,J., and Msadek,T. (2003) Comparative genomics reveal novel heat shock
678 regulatory mechanisms in Staphylococcus aureus and other Gram-positive bacteria. *Mol*
679 *Microbiol* **47:** 1061-1073.

680 Chien,Y., Manna,A.C., and Cheung,A.L. (1998) SarA level is a determinant of agr activation
681 in Staphylococcus aureus. *Mol Microbiol* **30:** 991-1001.

682 Clements,M.O., Watson,S.P., and Foster,S.J. (1999) Characterization of the major superoxide
683 dismutase of Staphylococcus aureus and its role in starvation survival, stress resistance, and
684 pathogenicity. *J Bacteriol* **181:** 3898-3903.

685 Cotter,P.D. and Hill,C. (2003) Surviving the acid test: responses of gram-positive bacteria to
686 low pH. *Microbiol Mol Biol Rev* **67:** 429-453.

687 Cremonesi,P., Perez,G., Pisoni,G., Moroni,P., Morandi,S., Luzzana,M. et al. (2007) Detection
688 of enterotoxigenic Staphylococcus aureus isolates in raw milk cheese. *Letters in Applied*
689 *Microbiology* **45:** 586-591.

690 Cui,L., Lian,J.Q., Neoh,H.m., Reyes,E., and Hiramatsu,K. (2005) DNA Microarray-Based
691 Identification of Genes Associated with Glycopeptide Resistance in Staphylococcus aureus.
692 *Antimicrob Agents Chemother* **49:** 3404-3413.

693 De Buyser,M.L., Dufour,B., Maire,M., and Lafarge,V. (2001) Implication of milk and milk
694 products in food-borne diseases in France and in different industrialised countries. *Int J Food*
695 *Microbiol* **67:** 1-17.

For Peer Review Only

696 Delbes,C., Alomar,J., Chougui,N., Martin,J.F., and Montel,M.C. (2006) Staphylococcus
697 aureus growth and enterotoxin production during the manufacture of uncooked, semihard
698 cheese from cows' raw milk. *J Food Prot* **69:** 2161-2167.

699 Derzelle,S., Dilasser,F., Duquenne,M., and Deperrois,V. (2009) Differential temporal
700 expression of the staphylococcal enterotoxins genes during cell growth. *Food Microbiology*
701 **26:** 896-904.

702 Domenech,A., Hernandez,F.J., Orden,J.A., Goyache,J., Lopez,B., Suarez,G., and Gomez-
703 Lucia,E. (1992) Effect of six organic acids on staphylococcal growth and enterotoxin
704 production. *Zeitschrift fÇ¬r Lebensmitteluntersuchung und -Forschung A* **V194:** 124-128.

705 Dunman,P.M., Murphy,E., Haney,S., Palacios,D., Tucker-Kellogg,G., Wu,S. et al. (2001)
706 Transcription Profiling-Based Identification of Staphylococcus aureus Genes Regulated by
707 the agr and/or sarA Loci. *J Bacteriol* **183:** 7341-7353.

708 Duquenne,M., Fleurot,I., Aigle,M., Darrigo,C., Borezee-Durant,E., Derzelle,S. et al. (2010)
709 Tool for Quantification of Staphylococcal Enterotoxin Gene Expression in Cheese. *Applied*
710 *and Environmental Microbiology* **76:** 1367-1374.

711 Even,S., Charlier,C., Nouaille,S., Ben Zakour,N.L., Cretenet,M., Cousin,F.J. et al. (2009)
712 Staphylococcus aureus virulence expression is impaired by Lactococcus lactis in mixed
713 cultures. *Appl Environ Microbiol* **75:** 4459-4472.

714 Fuchs,S., Pane-Farre,J., Kohler,C., Hecker,M., and Engelmann,S. (2007) Anaerobic gene
715 expression in Staphylococcus aureus. *J Bacteriol* **189:** 4275-4289.

For Peer Review Only

716 Garzoni,C., Francois,P., Huyghe,A., Couzinet,S., Tapparel,C., Charbonnier,Y. et al. (2007) A
717 global view of Staphylococcus aureus whole genome expression upon internalization in
718 human epithelial cells. *BMC Genomics* **8:** 171.

719 Geiger,T., Goerke,C., Mainiero,M., Kraus,D., and Wolz,C. (2008) The virulence regulator
720 Sae of Staphylococcus aureus: promoter activities and response to phagocytosis-related
721 signals. *J Bacteriol* **190:** 3419-3428.

722 Geisinger,E., Adhikari,R.P., Jin,R., Ross,H.F., and Novick,R.P. (2006) Inhibition of rot
723 translation by RNAIII, a key feature of agr function. *Mol Microbiol* **61:** 1038-1048.

724 Genigeorgis,C., Foda,M.S., Mantis,A., and Sadler,W.W. (1971) Effect of sodium chloride and
725 pH on enterotoxin C production. *Appl Microbiol* **21:** 862-866.

726 Haines,W.C. and Harmon,L.G. (1973) Effect of selected lactic acid bacteria on growth of
727 Staphylococcus aureus and production of enterotoxin. *Appl Microbiol* **25:** 436-441.

728 Hennekinne,J.A., Guillier,F., Perelle,S., De Buyser,M.L., Dragacci,S., Krys,S., and
729 Lombard,B. (2007) Intralaboratory validation according to the EN ISO 16 140 standard of the
730 Vidas SET2 detection kit for use in official controls of staphylococcal enterotoxins in milk
731 products. *J Appl Microbiol* **102:** 1261-1272.

732 Hiron,A., Borezee-Durant,E., Piard,J.C., and Juillard,V. (2007) Only one of four oligopeptide
733 transport systems mediates nitrogen nutrition in Staphylococcus aureus. *J Bacteriol* **189:**
734 5119-5129.

735 Ikeda,T., Tamate,N., Yamaguchi,K., and Makino,S. (2005) Mass Outbreak of Food Poisoning
736 Disease Caused by Small Amounts of Staphylococcal Enterotoxins A and H. *Appl Environ*
737 *Microbiol* **71:** 2793-2795.

For Peer Review Only

738 Ingavale,S., van,W.W., Luong,T.T., Lee,C.Y., and Cheung,A.L. (2005) Rat/MgrA, a regulator
739 of autolysis, is a regulator of virulence genes in Staphylococcus aureus. *Infect Immun* **73:**
740 1423-1431.

741 Jorgensen,H.J., Mork,T., and Rorvik,L.M. (2005) The occurrence of Staphylococcus aureus
742 on a farm with small-scale production of raw milk cheese. *J Dairy Sci* **88:** 3810-3817.

743 Lammers,A., Kruijt,E., van de Kuijt,C., Nuijten,P.J., and Smith,H.E. (2000) Identification of
744 Staphylococcus aureus genes expressed during growth in milk: a useful model for selection of
745 genes important in bovine mastitis? *Microbiology* **146 (Pt 4):** 981-987.

746 Laughton,J.M., Devillard,E., Heinrichs,D.E., Reid,G., and McCormick,J.K. (2006) Inhibition
747 of expression of a staphylococcal superantigen-like protein by a soluble factor from
748 Lactobacillus reuteri. *Microbiology* **152:** 1155-1167.

749 Le Loir,Y., Baron,F., and Gautier,M. (2003) Staphylococcus aureus and food poisoning.
750 *Genet Mol Res* **2:** 63-76.

751 Lehn,N., Schaller,E., Wagner,H., and Kronke,M. (1995) Frequency of Toxic Shock Syndrome
752 Toxin-Producing and Enterotoxin-Producing Clinical Isolates of Staphylococcus aureus.
753 *European Journal of Clinical Microbiology & Infectious Diseases* **14:** 43-46.

754 Mah,R.A., Fung,D.Y., and Morse,S.A. (1967) Nutritional requirements of Staphylococcus
755 aureus S-6. *Appl Microbiol* **15:** 866-870.

756 Majerczyk,C.D., Sadykov,M.R., Luong,T.T., Lee,C., Somerville,G.A., and Sonenshein,A.L.
757 (2008) Staphylococcus aureus CodY negatively regulates virulence gene expression. *J*
758 *Bacteriol* **190:** 2257-2265.

For Peer Review Only

759 Mann,E.E., Rice,K.C., Boles,B.R., Endres,J.L., Ranjit,D., Chandramohan,L. et al. (2009)
760 Modulation of eDNA release and degradation affects Staphylococcus aureus biofilm
761 maturation. *PLoS One* **4:** e5822.

762 Marcos,A. (1993) Water activity in cheese in relation to composition, stability and safety. In
763 *Cheese: chemistry, physics and microbiology*. Fox,P.F. (ed). London: Chapman & Hall, pp.
764 439-469.

765 Mashburn,L.M., Jett,A.M., Akins,D.R., and Whiteley,M. (2005) Staphylococcus aureus
766 serves as an iron source for Pseudomonas aeruginosa during in vivo coculture. *J Bacteriol*
767 **187:** 554-566.

768 Maubois J.L. (1997) La preparation et la mise en oeuvre des rétentats d'ultrafiltration.
769 L'emploi du procédé MMV. In *Le fromage*. Eck,A. and Gillis,J.C. (eds). Paris: Technique &
770 Documentation, pp. 319-323.

771 Meyrand,A., Boutrand-Loei,S., Ray-Gueniot,S., Mazuy,C., Gaspard,C.E., Jaubert,G. et al.
772 (1998) Growth and enterotoxin production of Staphylococcus aureus during the manufacture
773 and ripening of Camembert-type cheeses from raw goats' milk. *J Appl Microbiol* **85:** 537-544.

774 Mistry V.V. and Maubois J.L. (1993) Application of membrane separation technology to
775 cheese production. In *Cheese: chemistry, physics and microbiology*. Fox,P.F. (ed). London:
776 Chapman & Hall, pp. 493-522.

777 Mossel,D.A.A. and Van Netten,P. (1990) Staphylococcus aureus and Related Staphylococci
778 in Foods - Ecology, Proliferation, Toxinogenesis, Control and Monitoring. *Journal of Applied*
779 *Bacteriology* **69:** S123-S145.

For Peer Review Only

780 Notermans,S. and Heuvelman,C.J. (1983) Combined effect of water activity, pH and sub-
781 optimal temperature on growth and enterotoxin production of Staphylococcus aureus. *Journal
782 of Food Science* **48:** 1832-1835.

783 Nouaille,S., Even,S., Charlier,C., Le,L.Y., Cocaign-Bousquet,M., and Loubiere,P. (2009)
784 Transcriptomic response of Lactococcus lactis in mixed culture with Staphylococcus aureus.
785 *Appl Environ Microbiol* **75:** 4473-4482.

786 Novick,R.P. (2003) Autoinduction and signal transduction in the regulation of staphylococcal
787 virulence. *Mol Microbiol* **48:** 1429-1449.

788 Omoe,K., Hu,D.L., Takahashi-Omoe,H., Nakane,A., and Shinagawa,K. (2005)
789 Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin
790 genes in Staphylococcus aureus isolates. *FEMS Microbiol Lett* **246:** 191-198.

791 Orwin,P.M., Leung,D.Y., Donahue,H.L., Novick,R.P., and Schlievert,P.M. (2001)
792 Biochemical and biological properties of Staphylococcal enterotoxin K. *Infect Immun* **69:**
793 360-366.

794 Otero,A., Garcia,M., Garcia,M., Santos,J., and Moreno,B. (1993) Behaviour of
795 Staphylococcus aureus strains FRI 137 and FRI 361 during the manufacture and ripening of
796 Manchego cheese. *International Dairy Journal* **3:** 85-96.

797 Otero,A., Garcia,M.L., Garcia,M.C., Moreno,B., and Bergdoll,M.S. (1990) Production of
798 staphylococcal enterotoxins C1 and C2 and thermonuclease throughout the growth cycle.
799 *Appl Environ Microbiol* **56:** 555-559.

For Peer Review Only

800 Otero,M.C. and Nader-Macias,M.E. (2006) Inhibition of Staphylococcus aureus by H2O2-
801 producing Lactobacillus gasseri isolated from the vaginal tract of cattle. *Anim Reprod Sci* **96:**
802 35-46.

803 Pane-Farre,J., Jonas,B., Forstner,K., Engelmann,S., and Hecker,M. (2006) The sigmaB
804 regulon in Staphylococcus aureus and its regulation. *Int J Med Microbiol* **296:** 237-258.

805 Pane-Farre,J., Jonas,B., Hardwick,S.W., Gronau,K., Lewis,R.J., Hecker,M., and
806 Engelmann,S. (2009) Role of RsbU in controlling SigB activity in Staphylococcus aureus
807 following alkaline stress. *J Bacteriol* **191:** 2561-2573.

808 Pohl,K., Francois,P., Stenz,L., Schlink,F., Geiger,T., Herbert,S. et al. (2009) CodY in
809 Staphylococcus aureus: a regulatory link between metabolism and virulence gene expression.
810 *J Bacteriol* **191:** 2953-2963.

811 Poli,A., Guglielmini,E., Sembeni,S., Spiazzi,M., Dellaglio,F., Rossi,F., and Torriani,S. (2007)
812 Detection of Staphylococcus aureus and enterotoxin genotype diversity in Monte Veronese, a
813 Protected Designation of Origin Italian cheese. *Letters in Applied Microbiology* **45:** 529-534.

814 R Development Core Team . R: A Language and Environment for Statistical Computing.
815 2007. R Foundation for Statistical Computing.
816 Ref Type: Computer Program

817 Regassa,L.B. and Betley,M.J. (1993) High sodium chloride concentrations inhibit
818 staphylococcal enterotoxin C gene (sec) expression at the level of sec mRNA. *Infect Immun*
819 **61:** 1581-1585.

For Peer Review Only

820 Regassa,L.B., Novick,R.P., and Betley,M.J. (1992) Glucose and nonmaintained pH decrease
821 expression of the accessory gene regulator (agr) in Staphylococcus aureus. *Infect Immun* **60:**
822 3381-3388.

823 Resch,A., Rosenstein,R., Nerz,C., and Gotz,F. (2005) Differential Gene Expression Profiling
824 of Staphylococcus aureus Cultivated under Biofilm and Planktonic Conditions. *Appl Environ*
825 *Microbiol* **71:** 2663-2676.

826 Rice,K.C., Firek,B.A., Nelson,J.B., Yang,S.J., Patton,T.G., and Bayles,K.W. (2003) The
827 Staphylococcus aureus cidAB operon: Evaluation of its role in regulation of murein hydrolase
828 activity and penicillin tolerance. *Journal of Bacteriology* **185:** 2635-2643.

829 Rice,K.C., Mann,E.E., Endres,J.L., Weiss,E.C., Cassat,J.E., Smeltzer,M.S., and Bayles,K.W.
830 (2007) The cidA murein hydrolase regulator contributes to DNA release and biofilm
831 development in Staphylococcus aureus. *Proc Natl Acad Sci U S A* **104:** 8113-8118.

832 Rice,K.C., Nelson,J.B., Patton,T.G., Yang,S.J., and Bayles,K.W. (2005) Acetic acid induces
833 expression of the Staphylococcus aureus cidABC and lrgAB murein hydrolase regulator
834 operons. *Journal of Bacteriology* **187:** 813-821.

835 Rogasch,K., Ruhmling,V., Pane-Farre,J., Hoper,D., Weinberg,C., Fuchs,S. et al. (2006)
836 Influence of the two-component system SaeRS on global gene expression in two different
837 Staphylococcus aureus strains. *J Bacteriol* **188:** 7742-7758.

838 Savijoki,K., Ingmer,H., and Varmanen,P. (2006) Proteolytic systems of lactic acid bacteria.
839 *Applied Microbiology and Biotechnology* **71:** 394-406.

For Peer Review Only

840 Schmid,R., Uhlemann,E.M., Nolden,L., Wersch,G., Hecker,R., Hermann,T. et al. (2000)
841 Response to nitrogen starvation in Corynebacterium glutamicum. *FEMS Microbiology Letters*
842 **187:** 83-88.

843 Senn,M.M., Giachino,P., Homerova,D., Steinhuber,A., Strassner,J., Kormanec,J. et al. (2005)
844 Molecular analysis and organization of the sigmaB operon in Staphylococcus aureus. *J*
845 *Bacteriol* **187:** 8006-8019.

846 Sharer,M.V., Su,C., Hegde,N.V., Jayarao,B.M., and Sordillo,L.M. (2003) Differential
847 expression of the lactose transporter gene affects growth of Staphylococcus aureus in milk. *J*
848 *Dairy Sci* **86:** 2373-81.

849 Somerville,G.A. and Proctor,R.A. (2009) At the crossroads of bacterial metabolism and
850 virulence factor synthesis in Staphylococci. *Microbiol Mol Biol Rev* **73:** 233-248.

851 Tachon,S., Brandsma,J.B., and Yvon,M. (2010) NoxE NADH Oxidase and the Electron
852 Transport Chain Are Responsible for the Ability of Lactococcus lactis To Decrease the Redox
853 Potential of Milk. *Applied and Environmental Microbiology* **76:** 1311-1319.

854 Thomas,D., Chou,S., Dauwalder,O., and Lina,G. (2007) Diversity in Staphylococcus aureus
855 enterotoxins. *Chem Immunol Allergy* **93:** 24-41.

856 Tirado,C. and Schmidt,K. (2001) WHO Surveillance Programme for Control of Foodborne
857 Infections and Intoxications: Preliminary Results and Trends Across Greater Europe. *Journal*
858 *of Infection* **43:** 80-84.

859 Ulve,V.M., Monnet,C., Valence,F., Fauquant,J., Falentin,H., and Lortal,S. (2008) RNA
860 extraction from cheese for analysis of in situ gene expression of Lactococcus lactis. *J Appl*
861 *Microbiol* **105:** 1327-1333.

For Peer Review Only

862 Vernozy-Rozand,C., Meyrand,A., Mazuy,C., ignette-Muller,M.L., Jaubert,G., Perrin,G. et al.
863 (1998) Behaviour and enterotoxin production by Staphylococcus aureus during the
864 manufacture and ripening of raw goats' milk lactic cheeses. *Journal of Dairy Research* **65:**
865 273-281.

866 Voyich,J.M., Vuong,C., DeWald,M., Nygaard,T.K., Kocianova,S., Griffith,S. et al. (2009)
867 The SaeR/S Gene Regulatory System Is Essential for Innate Immune Evasion by
868 Staphylococcus aureus. *Journal of Infectious Diseases* **199:** 1698-1706.

869 Wallin-Carlquist,N., Cao,R., Marta,D., Sant'ana da,S.A., Schelin,J., and Radstrom,P. (2010)
870 Acetic acid increases the phage-encoded enterotoxin A expression in Staphylococcus aureus.
871 *BMC Microbiol* **10:** 147.

872 Weinrick,B., Dunman,P.M., McAleese,F., Murphy,E., Projan,S.J., Fang,Y., and Novick,R.P.
873 (2004) Effect of mild acid on gene expression in Staphylococcus aureus. *J Bacteriol* **186:**
874 8407-8423.

875 Wertheim,H.F., Melles,D.C., Vos,M.C., van,L.W., van,B.A., Verbrugh,H.A., and
876 Nouwen,J.L. (2005) The role of nasal carriage in Staphylococcus aureus infections. *Lancet*
877 *Infect Dis* **5:** 751-762.

878 Xiong,Y.Q., van,W.W., Nast,C.C., Yeaman,M.R., Cheung,A.L., and Bayer,A.S. (2002)
879 Activation and transcriptional interaction between agr RNAII and RNAIII in Staphylococcus
880 aureus in vitro and in an experimental endocarditis model. *J Infect Dis* **186:** 668-677.

881 Yang,S.J., Dunman,P.M., Projan,S.J., and Bayles,K.W. (2006) Characterization of the
882 Staphylococcus aureus CidR regulon: elucidation of a novel role for acetoin metabolism in
883 cell death and lysis. *Molecular Microbiology* **60:** 458-468.

For Peer Review Only

884 Zhang,S., Iandolo,J.J., and Stewart,G.C. (1998) The enterotoxin D plasmid of Staphylococcus
885 aureus encodes a second enterotoxin determinant (sej). *FEMS Microbiol Lett* **168:** 227-33.
886

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887 **Table 1**: Expression profiles of selected genes determined by RT-qPCR. The average gene expression of three independent biological replicates

888 is expressed relative to *gyrB* x10. Statistically differentially expressed genes are in bold.

MW2 ORF	**Gene name**	**Description of gene product**	**Gene expression in pure culture**					**Gene expression in mixed culture**					**Ratio of expression Mixed/Pure**				
			6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d
Cell division																	
MW1069	*ftsZ*	cell division protein	68.1	60.9	59.8	40.3	74.5	**57.3**	**53.9**	**28.4**	**16.9**	**14.6**	0.8	0.9	**0.5**	**0.4**	**0.2**
MW0604	*pbp4*	Penicillin-binding protein 4	**2.9**	**1.8**	**1.6**	**1.0**	**3.3**	**4.0**	**4.7**	**2.8**	**1.1**	**0.3**	1.4	2.7	1.8	1.1	**0.1**
Stress response																	
MW1532	*dnaK*	DnaK protein	40.9	26.0	26.2	15.8	19.2	**55.8**	**43.5**	**60.7**	**132.7**	**438.6**	1.4	**1.7**	2.3	**8.4**	**22.9**
MW0477	*ctsR*	transcription repressor of stress genes	5.5	6.9	8.8	9.0	6.1	**7.7**	**7.0**	**9.5**	**46.1**	**149.0**	1.4	1.0	1.1	**5.1**	**24.3**
MW2212	*ureD*	urease accessory protein	**8.5**	**5.5**	**3.5**	**1.2**	**1.8**	**9.6**	**13.9**	**15.0**	**13.3**	**35.1**	1.1	**2.5**	**4.3**	**11.4**	**19.9**
MW2553	*arcC*	carbamate kinase	**1.6**	**7.7**	**15.6**	**0.6**	**6.5**	5.7	15.3	14.5	8.5	27.5	3.5	2.0	0.9	**13.4**	4.3
MW2552	*arcR*	deaminase pathway repressor	**2.3**	**9.0**	**16.6**	**1.2**	**8.3**	10.0	21.5	21.4	13.0	39.0	**4.4**	2.4	1.3	**10.8**	**4.7**
MW1505	*sodA*	superoxide dismutase	**122.0**	**189.5**	**209.6**	**137.8**	**68.2**	**135.8**	**178.4**	**157.8**	**506.3**	**288.3**	1.1	0.9	0.8	**3.7**	**4.2**
MW1028	*trxA*	thioredoxin	75.3	86.6	94.9	57.7	64.0	**104.0**	**108.0**	**64.5**	**39.3**	**25.5**	1.4	1.2	0.7	0.7	**0.4**
MW1221	*katA*	catalase	**14.4**	**31.2**	**51.6**	**37.4**	**26.2**	**16.7**	**27.5**	**26.3**	**20.2**	**5.5**	1.2	0.9	0.5	0.5	**0.2**
MW1988	*sigB*	sigma factor B	33.2	32.4	33.9	31.0	59.4	**26.4**	**43.4**	**33.9**	**31.4**	**12.8**	0.8	**1.3**	1.0	1.0	**0.2**
MW2108	*asp23*	alkaline shock protein 23	**428.4**	**1079**	**1331**	**1013**	**1239**	**206.8**	**300.4**	**423.5**	**1016**	**794.0**	0.5	**0.3**	**0.3**	1.0	0.6
MW2482	*crtN*	staphyloxanthin synthase	12.7	12.5	13.7	9.6	20.3	6.2	8.0	8.8	4.4	4.7	0.5	0.6	0.6	0.5	**0.2**
MW2469	*clpL*	ATPdependent Clp proteinase chain	**83.0**	**165.2**	**255.2**	**46.9**	**103.7**	**104.7**	**82.3**	**182.4**	**353.2**	**791.7**	1.3	0.5	0.7	**7.5**	**7.6**
MW0480	*clpC*	ATPdependent Clp proteinase chain	13.5	13.4	11.7	14.7	14.0	**12.7**	**12.3**	**27.2**	**145.9**	**422.1**	0.9	0.9	**2.3**	**9.9**	**30.2**
MW0730	*clpP*	ATPdependent Clp protease	40.6	41.7	41.5	32.2	45.4	**59.9**	**57.9**	**42.5**	**34.8**	**21.0**	1.5	1.4	1.0	1.1	**0.5**
MW1290	*cspA*	cold shock protein A	22.2	16.5	23.3	12.4	9.4	**35.7**	**40.2**	**19.0**	**9.4**	**5.4**	1.6	2.4	0.8	0.8	0.6
MW2623	*cspB*	cold shock protein B	**238.0**	**147.5**	**130.5**	**25.9**	**45.2**	**248.7**	**214.1**	**131.6**	**64.5**	**43.1**	1.0	1.5	1.0	**2.5**	1.0
Virulence																	
MW1959	*hld (RNAIII)*	deltahemolysin	**86.8**	**494.5**	**1104**	**4243**	**3293**	94.5	233.8	285.8	164.9	189.5	1.1	0.5	0.3	**3.9E-02**	**0.1**
MW2586	*icaA*	intercellular adhesion protein A	**0.2**	**0.5**	**0.9**	**0.1**	**0.3**	0.04	0.04	0.01	ND	ND	0.2	**0.1**	**5.7E-03**	NC	NC
MW2559	*isaB*	immunodominant antigen B	21.4	35.0	56.9	57.7	11.1	**23.6**	**24.4**	**22.1**	**83.7**	**158.7**	1.1	0.7	0.4	1.5	**14.3**

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MW2 ORF	Gene name	Description of gene product	Gene expression in pure culture					Gene expression in mixed culture					Ratio of expression Mixed/Pure				
			6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d
MW0764	*clfA*	clumping factor A	**74.8**	**123.6**	**206.5**	**147.9**	**144.8**	**52.4**	**74.6**	**80.5**	**337.8**	**409.2**	0.7	0.6	**0.4**	2.3	2.8
MW0516	*sdrC*	SerAsp rich fibrinogenbinding,	0.1	0.2	0.1	0.1	0.1	**0.6**	**2.2**	**4.1**	**2.8**	**3.1**	**4.1**	**14.0**	**32.9**	**37.8**	**30.1**
MW0124	*cap8A*	capsular polysaccharide Cap8A	**19.5**	**46.7**	**64.6**	**55.8**	**26.8**	**10.8**	**11.6**	**20.5**	**66.2**	**13.9**	0.6	**0.2**	**0.3**	1.2	0.5
MW1885	*sak*	staphylokinase	**1.5**	**1.7**	**1.4**	**0.4**	**0.6**	**2.9**	**3.1**	**0.9**	**0.5**	**1.6**	**1.9**	**1.9**	0.6	1.2	2.6
MW1889	*sea*	enterotoxin A	**5.8**	**6.7**	**6.0**	**2.2**	**1.1**	**10.4**	**14.9**	**6.5**	**5.6**	**3.2**	1.8	**2.2**	1.1	2.5	2.9
MW1937	*seg2*	enterotoxin G	**3.4**	**4.1**	**4.9**	**1.8**	**2.2**	**2.9**	**3.6**	**1.9**	**0.6**	**0.5**	0.9	0.9	**0.4**	**0.4**	**0.2**
MW1938	*sek2*	enterotoxin K	**1.5**	**1.7**	**1.9**	**0.8**	**0.9**	**1.6**	**2.0**	**0.9**	**0.4**	**0.7**	1.1	1.2	**0.5**	**0.5**	0.8
MW0760	*sel2*	enterotoxin L	**2.5**	**2.8**	**3.6**	**1.2**	**1.5**	**4.1**	**5.5**	**3.0**	**2.6**	**3.8**	**1.7**	**1.9**	0.8	**2.1**	**2.6**
MW0051	*seh*	enterotoxin H	1.6	1.4	2.5	0.1	0.2	**2.7**	**3.3**	**2.1**	**1.8**	**4.2**	1.7	**2.3**	0.8	**12.7**	**17.2**
MW0759	*sec4*	enterotoxin C	3.1	3.4	3.7	1.8	4.6	4.1	7.7	4.6	1.9	2.1	1.3	2.3	1.3	1.1	0.4
Carbohydrate metabolism																	
MW2115	*lacG*	6phosphobetagalactosidase	**2.4**	**11.5**	**20.5**	**17.1**	**55.0**	**9.8**	**30.4**	**19.4**	**7.0**	**23.6**	**4.0**	2.7	0.9	**0.4**	0.4
MW1641	*pykA*	pyruvate kinase	25.8	24.5	29.8	15.1	24.7	**27.0**	**24.3**	**33.9**	**28.2**	**77.6**	1.0	1.0	1.1	**1.9**	**3.1**
MW0217	*lctE*	Llactate dehydrogenase	**93.4**	**120.2**	**217.7**	**4.2**	**73.9**	**71.4**	**73.3**	**13.4**	**3.6**	**8.7**	0.8	**0.6**	**0.1**	0.8	0.1
MW0568	*adh1*	alcohol dehydrogenase I	**18.3**	**25.3**	**41.7**	**0.5**	**4.9**	**15.4**	**19.8**	**3.5**	**1.2**	**3.6**	0.8	0.8	0.1	2.5	0.7
MW0202	*pflA*	formate acetyltransferase enzyme	**65.5**	**122.7**	**125.4**	**3.6**	**20.0**	**176.7**	**269.7**	**73.1**	**28.2**	**86.1**	**2.7**	**2.2**	0.6	**7.9**	**4.3**
MW1237	*citb*	aconitate hydratase	**12.4**	**34.2**	**47.1**	**27.0**	**18.2**	**15.2**	**32.0**	**28.0**	**46.0**	**7.6**	1.2	0.9	0.6	1.7	**0.4**
MW2526	*mqo2*	malate/quinone oxidoreductase	48.1	36.3	31.8	25.9	25.0	**42.1**	**36.0**	**28.0**	**23.5**	**38.8**	0.9	1.0	0.9	0.9	1.6
MW1129	*sucD*	succinylCoA synthetase	80.9	97.6	84.7	63.5	31.2	**87.0**	**89.7**	**38.2**	**25.6**	**18.1**	1.1	0.9	**0.5**	0.4	0.6
MW2460	*cidC*	pyruvate oxydase	12.9	20.6	21.4	26.6	33.6	**9.5**	**11.9**	**22.1**	**78.9**	**50.8**	0.7	**0.6**	1.0	**3.0**	1.5
MW2462	*cidA*	holin-like protein cidA	**0.1**	**0.1**	**0.1**	**0.1**	**0.3**	**0.5**	**1.5**	**2.3**	**4.8**	**3.3**	**3.9**	**14.2**	**19.4**	**79.0**	12.2
MW0238	*lrgA*	holin-like protein LrgA	0.4	0.5	0.5	0.1	1.0	**2.4**	**1.1**	**0.5**	**0.3**	**0.9**	**5.3**	2.2	1.0	**2.0**	1.0
MW2132	*alsS*	alpha-acetolactate synthase	0.8	0.8	1.0	0.5	4.4	**2.9**	**8.5**	**15.6**	**48.0**	**87.9**	**3.6**	**11.2**	**15.2**	**89.2**	**20.1**
MW0100	*butA*	acetoin(diacetyl)reductase	9.2	13.5	20.8	24.7	14.5	**12.6**	**15.9**	**23.9**	**48.2**	**193.1**	1.4	1.2	1.2	**2.0**	**13.3**
Nitrogen metabolism																	
MW0705	*pepT*	aminotripeptidase	9.7	11.0	9.8	10.2	10.8	**9.9**	**9.9**	**7.2**	**4.2**	**5.1**	1.0	0.9	**0.7**	**0.4**	**0.5**
MW1694		XaaHis dipeptidase homolog	2.6	3.1	3.3	2.2	2.7	**3.4**	**4.2**	**3.8**	**1.3**	**0.9**	1.3	**1.3**	1.2	**0.6**	**0.3**
MW1584	*relA*	GTP pyrophosphokinase	6.0	7.5	7.7	4.1	7.6	**5.6**	**5.2**	**4.5**	**1.0**	**1.0**	0.9	**0.7**	0.6	**0.2**	**0.1**
MW1977	*ilvD*	dihydroxy-acid dehydratase	11.2	9.2	4.1	25.2	2.4	**46.0**	**4.7**	**2.2**	**0.2**	**1.1**	4.1	0.5	0.5	**7.8E-03**	**0.4**

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MW2 ORF	Gene name	Description of gene product	Gene expression in pure culture					Gene expression in mixed culture					Ratio of expression Mixed/Pure				
			6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d	6 h	8 h	10 h	24 h	7 d
MW1981	*leuA*	2-isopropylmalate synthase	16.6	15.0	13.0	34.5	3.8	**116.1**	**13.4**	**1.8**	**1.0**	**2.7**	7.0	0.9	0.1	**2.8E-02**	0.7
MW1138	*codY*	transcription pleiotropic repressor	24.0	23.8	24.3	13.7	30.1	**31.9**	**35.6**	**26.8**	**10.4**	**11.3**	1.3	**1.5**	1.1	0.8	**0.4**
Regulators																	
MW1963	*agrA (RNAII)*	accessory gene regulator A	**20.7**	**38.7**	**51.8**	**111.1**	**200.4**	**25.1**	**61.4**	**93.4**	**59.7**	**53.1**	1.2	1.6	**1.8**	0.5	**0.3**
MW0580	*sarA*	staphylococcal accessory regulator A	313.0	459.2	544.4	417.4	420.8	257.3	298.7	342.4	418.0	162.0	0.8	0.7	**0.6**	1.0	**0.4**
MW1705	*rot*	transcriptional regulator	**36.8**	**31.1**	**36.3**	**12.1**	**5.6**	**38.7**	**37.7**	**24.9**	**22.0**	**19.5**	1.1	1.2	0.7	1.8	**3.5**
MW0667	*saeS*	histidine protein kinase	**20.1**	**18.5**	**11.4**	**5.3**	**27.3**	**19.5**	**28.2**	**11.7**	**3.7**	**1.3**	1.0	1.5	1.0	0.7	**0.0**
MW0648	*mgrA*	transcriptional regulator	37.1	26.7	27.6	17.3	20.6	**48.1**	**32.0**	**18.1**	**12.2**	**9.4**	1.3	1.2	0.7	0.7	**0.5**
MW2585	*icaR*	operon ica repressor	**8.6**	**13.0**	**19.7**	**10.5**	**3.9**	**10.6**	**13.3**	**9.3**	**12.0**	**3.0**	1.2	1.0	0.5	1.1	0.8
MW1446	*srrA*	staphylococcal respiratory response	27.3	25.5	28.4	11.7	17.9	29.0	31.7	15.6	21.9	9.5	1.1	1.2	0.6	1.9	0.5

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890 **Table 2.** Expression ratio of SE genes and *saeS* in *S. aureus* MW2 strain grown in CDM
891 *versus* cheese matrix, as determined by RT-qPCR, using *gyrB* as an internal standard for
892 normalisation. Data on CDM were adapted from Even et al. (Even et al., 2009). Statistically
893 differentially expressed genes between CDM and cheese matrix are in bold (as determined by
894 T-test considering a P value lower than 0.05).

			Ratio of expression CDM/cheese matrix			
MW2 ORF	Gene name	Description of gene product	6 h - exponential phase	8 h - late exponential phase	10 h - entry into stationary phase	24 h - stationary phase
MW1889	*sea*	enterotoxin A	0.8	1.2	0.8	0.5
MW1937	*seg2*	enterotoxin G	1.4	**2.3**	1.2	1.4
MW1938	*sek2*	enterotoxin K	1.8	**2.7**	1.4	1.3
MW0760	*sel2*	enterotoxin L	**3.6**	**7.9**	**3.8**	**41.6**
MW0051	*seh*	enterotoxin H	**5.8**	**10.1**	**4.5**	**64.0**
MW0759	*sec4*	enterotoxin C	**3.3**	**11.6**	**15.1**	**22.8**
MW0667	*saeS*	histidine protein kinase	**0.5**	3.1	2.0	**7.6**

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912 **Figure legends**

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914 **Fig. 1**: Kinetics of growth of *S. aureus* MW2 in cheese matrix (▲) (A), lactose consumption
915 (●) lactate production (▼), acetate production (■) (B) and pH (◆) (C). Pure cultures are in
916 black. Mixed cultures are in white.

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918 **Fig. 2:** Gene expression (relative to *gyrB)* of *agrA* (RNAII) (A) and RNAIII (B) in *S. aureus*
919 MW2 wild-type and a *codY* mutant at 10 h, 24 h and 7 days of culture.

920

921 **Fig. 3**: *S. aureus* population (A), pH (B) and profiles of gene expression (relative to *gyrB)* of
922 *agrA* (RNAII) (C) and RNAIII (D) in pure culture of *S. aureus* (●), pure culture of *S. aureus*
923 with addition of GDL (○) and mixed culture with *L. lactis* (▼). Asterisks: significant change
924 in expression compared to pure culture as determined by T-test considering a P value lower
925 than 0.05.

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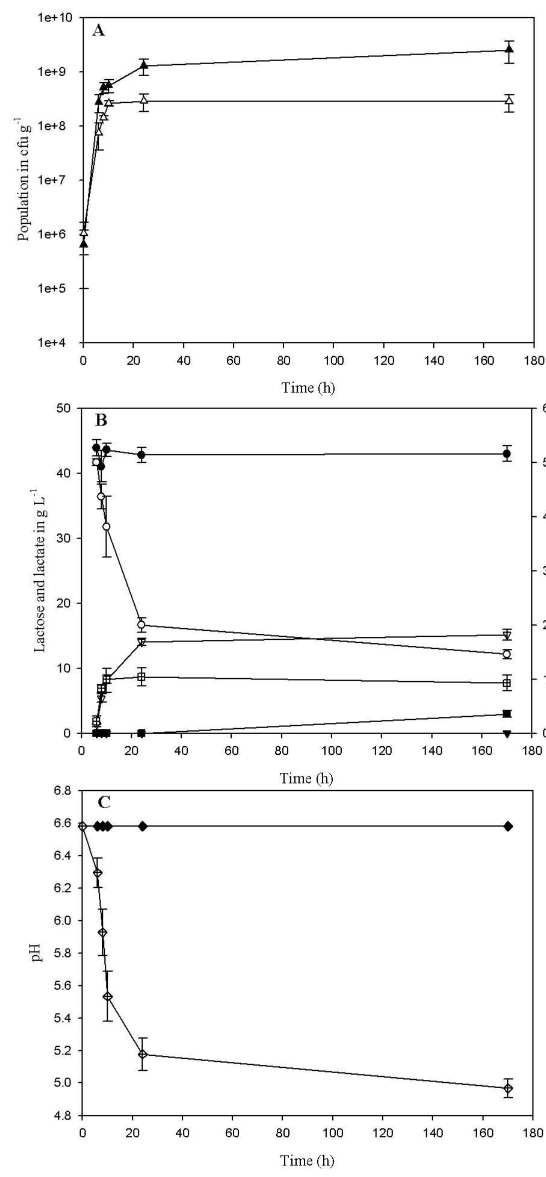

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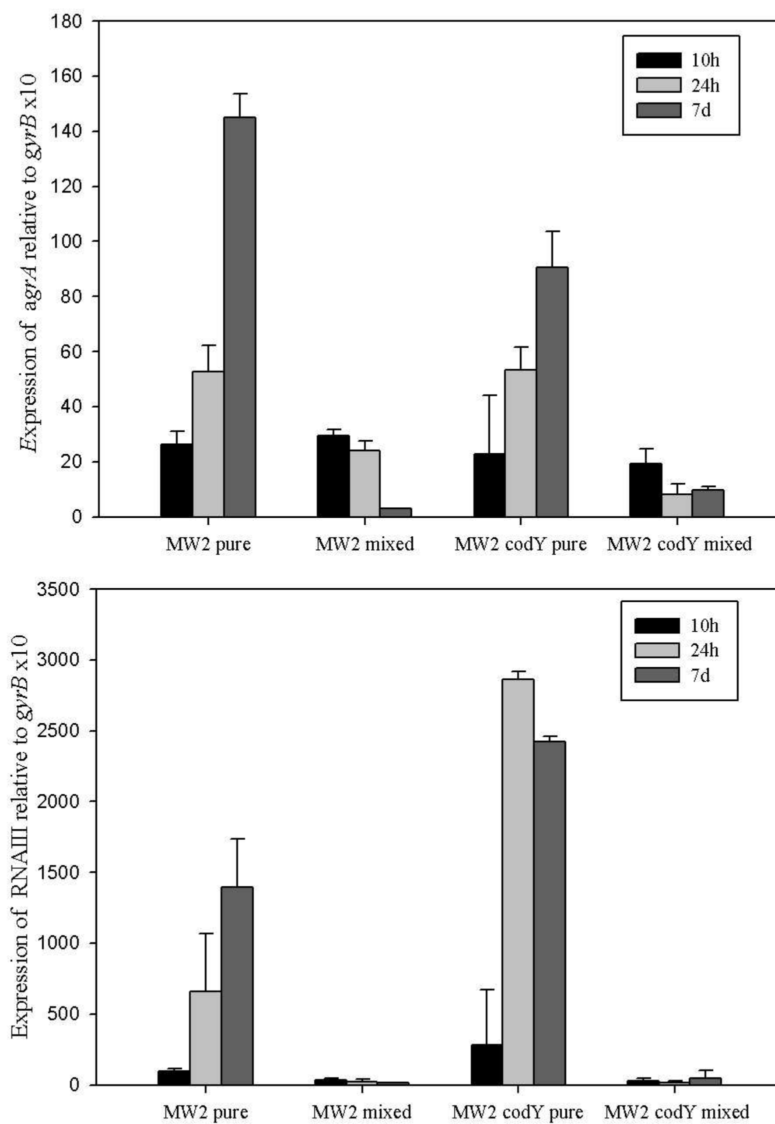

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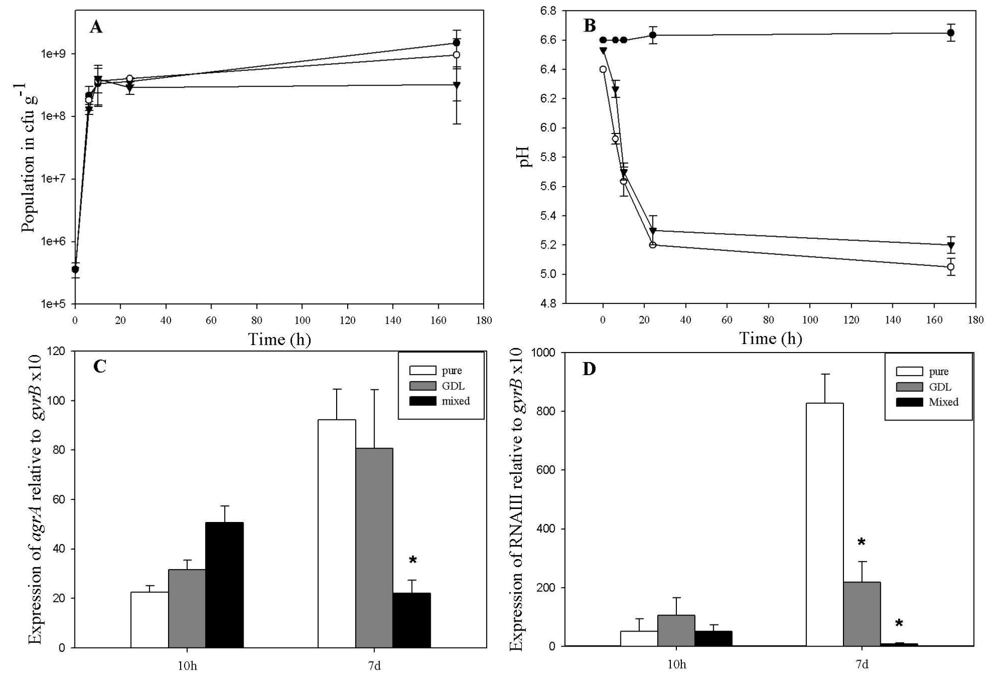

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Table S1. Expression profiles of *S. aureus* MW2 genes that exhibited significant variations in pure and/or mixed cultures with *L. lactis* LD61 in cheese matrix.

Expression levels in pure and mixed cultures at 6, 8, 10 and 24 h, detected with the *S. aureus* microarray and presented as normalized cDNA/gDNA ratios. Only genes that showed significant variations in expression levels in pure and or mixed cultures over time (as determined by the ANOVA test with the criterion of an FDR of 0.05) and a degree of change of 2-fold were defined as differentially expressed. Statistically differentially expressed genes are in bold.

MW2 ORF	Gene Name	Description of gene product	Gene expression in pure culture				Gene expression in mixed culture			
			6 h	8 h	10 h	24 h	6 h	8 h	10 h	24 h
DNA replication, recombination and repair										
MW1362	*hu*	DNAbinding protein II	54.3	86.8	84.3	70.6	**53.1**	**24.2**	**20.2**	**10.8**
MW1168	*recA*	RecA protein	9.3	9.5	11.1	9.9	**4.4**	**2.9**	**2.9**	**1.9**
MW0720	*uvrB*	exinuclease ABC subunit B	**0.7**	**0.7**	**0.7**	**1.4**	0.8	0.8	0.7	0.8
MW1029	*uvrC*	excinuclease ABC subunit C	**0.8**	**0.9**	**1.1**	**1.6**	1.2	1.4	1.4	1.3
MW1078	*tnp*	transposase	**0.9**	**1.0**	**1.1**	**2.5**	1.4	1.9	2.0	2.3
Transcription, translation and cellular division										
MW2045	*rho*	transcription termination factor Rho	2.0	1.5	1.3	1.3	**1.3**	**1.0**	**0.9**	**0.7**
MW0498	*rpoC*	RNA polymerase betaprime chain	6.0	3.1	2.1	6.7	**3.2**	**1.8**	**1.5**	**1.2**
MW1513	*sigA*	RNA polymerase sigma factor	**2.4**	**2.4**	**2.4**	**5.0**	1.6	1.4	1.9	2.0
MW1623	*rpmI*	50S ribosomal protein L35	9.3	6.0	4.0	3.9	**6.0**	**3.3**	**3.1**	**2.3**
MW0502	*fus*	translational elongation factor G	26.9	12.6	5.1	11.1	**13.8**	**5.3**	**3.8**	**1.8**
MW0503	*tufA*	translational elongation factor TU	44.1	24.2	11.8	20.0	**23.5**	**11.2**	**8.4**	**3.5**
IGS2		intergenic sequence 16S-23S	24.3	18.5	11.8	10.6	**13.1**	**7.4**	**6.5**	**3.4**
MW0466	*ftsH*	celldivision protein	6.1	6.3	5.7	8.2	**5.2**	**3.6**	**3.7**	**1.8**

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MW1063	*ftsL*	cell division protein	**5.1**	**5.6**	**3.4**	**2.0**	**2.5**	**1.7**	**1.4**	**0.7**
MW1069	*ftsZ*	cell division protein	5.9	4.3	5.0	5.3	**3.4**	**2.2**	**1.9**	**1.4**
MW2007		rod shape determining protein RodA	**4.1**	**14.3**	**25.5**	**106.1**	**2.6**	**3.0**	**4.8**	**2.3**
Cell wall biosynthesis and associated protein										
MW2589	*icaC*	intercellular adhesion protein C	**0.4**	**1.0**	**1.4**	**1.5**	0.7	0.6	0.6	0.9
MW1363	*gpsA*	glycerol3phosphate dehydrogenase	3.8	2.8	2.0	2.4	**2.2**	**1.6**	**1.3**	**1.0**
MW1064	*pbpA*	penicillinbinding protein 1	**2.2**	**1.6**	**1.2**	**0.9**	1.6	1.3	1.3	1.3
MW0604	*pbp4*	penicillin binding protein 4	4.2	2.4	1.3	2.0	**2.3**	**1.4**	**1.2**	**0.9**
MW0031	*mecA*	penicillin binding protein 2 prime	**0.5**	**0.7**	**1.2**	**1.3**	0.9	1.5	1.2	1.2
MW2013		lipoprotein precursor	4.6	3.6	1.6	2.8	**3.2**	**1.6**	**1.2**	**1.0**
Carbohydrate metabolism										
MW2115	*lacG*	6phosphobetagalactosidase	**0.6**	**1.7**	**3.1**	**4.6**	**1.2**	**1.6**	**1.7**	**1.0**
MW0217	*lctE*	Llactate dehydrogenase	**5.7**	**8.6**	**12.7**	**0.8**	**2.7**	**1.8**	**0.9**	**0.8**
MW0568	*adh1*	alcohol dehydrogenase I	**1.5**	**1.7**	**3.2**	**1.0**	1.1	1.0	0.8	0.9
MW1129	*sucD*	succinylCoA synthetase	13.7	17.4	16.5	19.7	**6.8**	**4.2**	**2.9**	**1.7**
MW2459	*ptsG*	PTS system, glucosespecific	**2.4**	**0.7**	**0.8**	**0.5**	0.6	0.5	0.5	0.5
MW2084	*mtlA*	PTS system, mannitol specific	**1.5**	**2.6**	**2.3**	**3.6**	1.0	0.8	1.0	1.3
MW0163	*glcA*	PTS enzyme II	**1.5**	**0.4**	**0.4**	**0.3**	0.5	0.7	0.6	0.8
MW0245	*rbsD*	ribose permease	**0.7**	**1.8**	**1.3**	**0.6**	**0.8**	**0.8**	**0.6**	**0.6**
MW0218		PTS enzyme, maltose and glucose specific	**0.8**	**0.5**	**0.7**	**0.3**	0.8	0.6	0.5	0.7
MW2538		similar to Mg citrate secondary transporter	3.3	2.1	1.9	1.5	**1.8**	**1.0**	**0.8**	**0.7**
MW2175		glucose uptake protein homolog	**1.1**	**0.7**	**0.5**	**0.7**	1.1	1.2	1.1	1.2
Nitrogen metabolism										
MW1980	*ilvC*	alphaketobetahydroxylacil reductoisomerase	5.6	6.3	1.2	5.7	**5.4**	**0.7**	**0.6**	**0.6**

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MW2304	*gltT*	proton/sodiumglutamate symport protein	7.0	5.4	4.1	7.0	**3.5**	**2.2**	**1.6**	**1.0**
MW1694		XaaHis dipeptidase homolog	**0.6**	**0.7**	**0.9**	**1.1**	**1.9**	**1.5**	**1.2**	**0.7**
MW0705	*pepT*	aminotripeptidase	**1.9**	**2.5**	**2.5**	**4.6**	1.2	1.0	1.0	1.1
MW2522		amino acid transporter	7.1	5.0	4.2	3.2	**3.3**	**1.2**	**1.0**	**0.6**
MW0875	*appF*	oligopeptide transport system ATPbinding protein AppF homologue	0.6	1.0	0.6	0.5	**3.1**	**2.3**	**1.3**	**1.0**
MW0868	*oppB*	oligopeptide transport system permease protein	6.1	5.9	6.1	4.2	**2.7**	**2.2**	**1.8**	**1.0**
MW0871	*opp3*	oligopeptide transport system ATPbinding protein OppF homologue	6.4	5.9	5.3	5.0	**2.4**	**1.8**	**1.3**	**1.0**
MW2553	*arcC*	carbamate kinase	**0.3**	**1.2**	**2.1**	**0.3**	0.7	0.9	1.1	0.8
MW1138	*codY*	transcription pleiotropic repressor	3.8	4.6	3.2	3.2	**2.4**	**1.7**	**1.6**	**0.9**
Energetic metabolism										
MW0999	*ctaB*	cytochrome caa3 oxidase homologue	4.2	4.4	4.9	3.1	**2.7**	**2.1**	**1.5**	**1.2**
MW0588		MnhD homologue,similar to Na+/H+ antiporter subunit	15.5	7.9	4.0	6.3	**7.3**	**3.6**	**2.7**	**1.6**
MW2526	*mqo2*	malate/quinone oxidoreductase	**3.1**	**1.5**	**1.7**	**1.9**	1.2	1.0	1.0	0.9
Vitamins, nucleotides and nucleic acid metabolism										
MW2536		anaerobic (class III) ribonucleotide reductase small subunit chain	**0.7**	**0.7**	**1.1**	**0.3**	0.7	0.8	0.9	0.9
MW2196	*modB*	probable molybdenum transport permease	**1.4**	**1.6**	**1.8**	**3.2**	0.5	0.6	0.7	0.7
MW2186	*moaA*	molybdenum cofactor biosynthesis protein A	**0.5**	**0.5**	**0.5**	**0.9**	**0.5**	**0.6**	**0.7**	**0.8**
MW0476	*nupC*	pyrimidine nucleoside transport protein	2.5	2.3	2.0	1.6	**2.0**	**1.4**	**1.0**	**0.8**
Iron metabolism										
MW1011	*isdB*	ironregulated cell wallanchored protein	0.4	0.4	0.6	0.6	**0.7**	**0.9**	**1.0**	**1.1**
MW1834		ferritin	0.9	4.6	8.6	5.4	**0.8**	**1.8**	**1.7**	**3.1**
MW0200		similar to periplasmicironbinding protein BitC	**0.5**	**0.9**	**0.9**	**1.2**	0.6	0.8	0.7	0.9
Stress response										

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MW1531	*dnaJ*	DnaJ protein	**3.9**	**2.2**	**1.8**	**2.0**	2.6	1.8	2.8	3.0
MW1532	*dnaK*	DnaK protein	5.5	5.0	4.0	4.6	**3.7**	**2.2**	**4.7**	**7.5**
MW0477	*ctsR*	transcription repressor of stress genes	1.2	1.6	2.0	3.3	**1.2**	**1.1**	**1.3**	**2.8**
MW2469	*clpL*	ATPdependent Clp proteinase chain	**6.5**	**11.9**	**15.5**	**5.3**	**4.0**	**1.9**	**6.5**	**7.2**
MW1136	*clpQ*	heat shock protein HslV	3.7	3.9	3.2	3.3	**2.8**	**2.0**	**1.8**	**1.3**
MW1618	*clpX*	protease	5.5	5.0	4.0	4.6	**2.3**	**1.5**	**1.5**	**1.6**
MW0726	*trxB*	thioredoxine reductase	5.7	5.5	6.0	5.8	**4.0**	**3.1**	**3.2**	**1.6**
MW2535	*cudT*	choline transporter	**0.3**	**0.4**	**0.5**	**1.1**	0.7	0.8	0.6	0.6
MW0356	*ahpF*	alkyl hydroperoxide reductase subunit F	**0.6**	**0.7**	**0.9**	**4.5**	1.0	0.9	1.0	1.1
MW1221	*katA*	catalase	**10.4**	**21.7**	**24.5**	**36.2**	9.9	9.6	13.8	9.1
MW2212	*ureD*	urease accessory protein	**1.2**	**0.7**	**0.6**	**0.4**	1.1	1.2	1.6	1.3
MW0453	*spoVG*	stage V sporulation protein G homologue	**1.4**	**2.3**	**4.1**	**6.5**	**0.9**	**0.9**	**1.3**	**1.5**
MW1991	*rsbU*	sigmaB regulation protein	4.1	3.5	2.8	2.9	**1.6**	**1.1**	**1.0**	**0.7**
MW2108	*asp23*	alkaline shock protein 23	**28.3**	**57.4**	**72.3**	**78.6**	**14.7**	**9.1**	**26.6**	**35.7**
MW1682		general stress proteinlike protein	**13.3**	**24.2**	**34.6**	**41.5**	6.6	4.4	8.4	7.8
MW0781		similar to general stress protein	**4.2**	**10.9**	**17.0**	**29.1**	**2.2**	**1.8**	**4.6**	**6.6**
Virulence										
MW1963	*agrA*	accessory gene regulator A	**1.4**	**2.3**	**3.3**	**10.8**	**1.2**	**1.5**	**3.0**	**1.5**
sRNA_RNAIII	RNAIII	RNAIII	**8.2**	**39.2**	**68.5**	**116.7**	**6.5**	**8.3**	**15.1**	**6.3**
MW0668	*saeR*	response regulator	4.5	4.3	3.9	2.6	**2.4**	**2.0**	**1.5**	**0.7**
MW2418	*sarH2*	staphylococcal accessory regulator A homolog	**0.2**	**0.3**	**0.5**	**1.1**	**0.5**	**0.7**	**0.8**	**1.0**
MW2420	*fnbB*	fibronectinbinding protein homolog	**0.5**	**0.3**	**0.2**	**0.2**	0.5	0.5	0.5	0.5
MW2421	*fnb*	fibrinogenbinding protein	0.5	0.3	0.2	0.2	**3.4**	**2.8**	**5.4**	**9.5**
MW0764	*fnb*	fibrinogenbinding protein	**6.0**	**12.7**	**16.2**	**18.3**	3.4	2.8	5.4	9.5
MW1369	*ebpS*	elastin binding protein	**2.1**	**3.5**	**5.8**	**7.0**	2.1	1.8	2.8	2.3
MW2559	*isaB*	immunodominant antigen B	**1.3**	**2.7**	**3.7**	**5.8**	**1.2**	**1.1**	**1.1**	**2.3**
MW0517	*sdrD*	SerAsp rich fibrinogenbinding	0.2	0.2	0.2	0.4	**0.4**	**0.6**	**0.6**	**0.9**

For Peer Review Only

MW0124	*cap8A*	capsular polysaccharide synthesis enzyme Cap5A	**4.0**	**10.7**	**12.2**	**15.9**	**1.6**	**1.3**	**2.3**	**4.1**
MW0139	*cap8P*	capsular polysaccharide synthesis enzyme Cap5P	**1.0**	**0.9**	**1.0**	**0.4**	**0.7**	**0.7**	**0.8**	**1.2**
MW0627		secretory antigen SsaA homologue	6.9	7.8	10.3	9.1	**3.8**	**3.6**	**3.4**	**1.8**
MW2589	*aur*	zinc metalloproteinase aureolysin	**3.0**	**3.0**	**1.4**	**0.9**	**2.2**	**1.7**	**0.9**	**0.9**
MW0802		hemolysin	**0.6**	**1.0**	**1.1**	**1.4**	**0.6**	**0.7**	**0.8**	**1.1**
MW2096		hemolysin III	**0.9**	**0.9**	**1.2**	**2.8**	0.9	0.9	0.9	1.0
MW2344	*hlgB*	gammahemolysin component B	**0.9**	**0.5**	**0.5**	**0.2**	0.7	0.7	0.6	0.6
Signal transduction/ secretion										
MW1775	*traP*	signal transduction protein	**3.9**	**4.2**	**4.4**	**2.5**	**1.9**	**1.4**	**1.3**	**1.0**
MW2149	*secY*	preprotein translocase SecY subunit	45.0	20.5	10.7	26.4	**21.6**	**8.8**	**6.0**	**3.2**
Transporters										
MW1235	*mscL*	largeconductance mechanosensitive channel	6.8	7.0	10.2	9.5	**3.1**	**2.3**	**1.9**	**1.4**
MW0988		Mn2+transport protein	3.1	2.8	3.8	4.5	**13.3**	**16.9**	**11.1**	**4.5**
MW1507		ABC transporter MreB	1.1	0.8	0.7	0.8	**1.0**	**0.9**	**1.1**	**1.5**
MW1554		probable transmembrane transport protein	0.4	0.5	0.5	0.7	**0.4**	**0.5**	**0.5**	**0.7**
MW2298		cationic transporter	**0.2**	**0.6**	**0.6**	**1.5**	0.4	0.5	0.4	0.5
MW0586		Na+/H+ antiporter	**5.8**	**2.2**	**1.4**	**2.7**	3.0	1.6	1.6	1.2
MW0605		ATPbinding cassette transporter A	**1.2**	**1.2**	**1.3**	**3.3**	2.6	2.3	3.6	3.1
MW2478	*copA*	coppertransporting ATPase	0.5	0.5	0.4	1.0	**0.5**	**0.6**	**0.9**	**1.7**
MW2334		ABC transporter (ATP binding subunit)	**0.5**	**0.3**	**0.4**	**0.4**	0.6	0.7	0.7	0.8
MW0213		similar to nickel ABC transporter nickelbinding protein	**0.4**	**0.3**	**0.4**	**0.2**	0.6	0.6	0.6	0.6
Regulators										
MW1446	*srrA*	staphylococcal respiratory response protein	3.4	3.3	2.8	2.4	**2.2**	**1.6**	**1.3**	**1.2**
MW1445	*srrB*	staphylococcal respiratory response protein	**1.1**	**0.9**	**0.8**	**0.6**	1.1	1.0	1.1	1.1
MW1790	*yhcS*	two-component sensor histidine kinase	**0.6**	**0.7**	**0.9**	**2.0**	0.6	0.6	0.7	0.8
MW1789	*yhcR*	two-component response regulator homolog	0.6	0.7	0.8	0.9	**0.9**	**1.0**	**1.3**	**1.5**

For Peer Review Only

MW0018	*vicR*	response regulator	**1.9**	**3.1**	**3.5**	**2.7**	1.7	1.5	1.8	2.0
MW1637	*phoP*	alkaline phosphatase synthesis transcriptional regulatory protein	**1.6**	**3.1**	**3.4**	**3.5**	1.4	1.3	1.5	1.7
MW1801	*perR*	transcription regulator Fur family homolog	**1.3**	**1.5**	**2.1**	**4.5**	1.1	1.0	1.0	1.0
MW0879	*spx*	conserved hypothetical protein	**8.0**	**10.2**	**16.2**	**24.3**	4.5	2.9	3.7	3.1
MW1111		transcription factor fapR	**3.4**	**3.0**	**2.0**	**1.4**	**2.0**	**1.4**	**1.1**	**0.9**
MW2552		transcriptional regulator	**0.4**	**1.0**	**2.1**	**1.0**	0.9	1.1	1.4	1.0
Antibiotic related genes										
MW0623	*vraF*	ABC transporter ATPbinding protein	**1.2**	**1.3**	**1.3**	**0.7**	0.9	0.9	0.7	0.9
MW1158		similar to metallobetalactamase family protein	**4.3**	**3.4**	**3.2**	**2.2**	2.2	1.4	1.6	1.2
Others										
sRNA_sgrA	sgrA		**1.3**	**2.0**	**2.9**	**5.1**	2.6	2.6	3.8	4.5
sRNA_sbrD	sbrD		**0.2**	**0.2**	**0.3**	**0.6**	0.4	0.5	0.7	0.9
sRNA_45S	45S		0.2	0.3	0.5	0.5	**1.6**	**1.6**	**2.9**	**2.8**
MW1494		exogenous DNAbinding protein comGC	7.6	8.9	9.4	10.1	**5.9**	**3.0**	**2.3**	**2.0**
MW0906		competence transcription factor	0.1	0.2	0.2	0.2	**0.5**	**0.6**	**0.7**	**0.8**
MW1619	*tig*	prolyl isomerase	3.7	2.5	2.0	1.9	**3.8**	**2.3**	**1.9**	**1.7**

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Table S2: Quantification of amino acids and urea in cheese matrix inoculated by *S. aureus* MW2, *L. lactis* LD61 or both. Concentrations are expressed in mmol g^{-1}.

Amino acids	Initial	6 h			8 h			10 h			24 h			7 d		
		L. lactis	Mixed	*S. aureus*	*L. lactis*	Mixed	*S. aureus*	*L. lactis*	Mixed	*S. aureus*	*L. lactis*	Mixed	*S. aureus*	*L. lactis*	Mixed	*S. aureus*
Urea	568.1	658.9	540.5	571.7	575.9	353.9	431.9	604.3	181.8	369.0	523.1		253.1	501.2	-	-
Asp	1.9	8.2	11.4	1.8	17.8	37.6	2.1	30.2	34.3	2.1	55.2	62.9	1.3	65.7	81.7	4.8
Thr	-	-	-	-	-	-	-	2.5	19.3	-	83.9	139.9	-	281.8	383.8	-
Ser	-	-	-	-	-	2.1	0.9	4.5	15.3	-	58.6	73.3	-	196.5	160.1	-
asn	-	-	-	-	-			-	4.4	-	32.6	61.3	-	141.7	208.6	-
Glu	47.2	64.3	47.4	15.2	61.8	94.9	7.3	119.5	212.2	15.6	496.4	569.2	6.2	849.1	974.0	39.8
Gln				-			-	-	9.6	-	33.1	55.6	-	253.7	242.2	-
Gly	13.9	16.9	3.5	-	5.4	6.4	-	9.4	19.4	-	79.3	106.8	-	126.2	193.4	-
Ala	4.4	6.7	8.0	-	10.7	16.6	-	24.0	43.4	-	131.6	180.3	-	340.7	430.9	3.9
Cit	-	-	-	-	-	7.2	-	13.3	15.2	-	37.0	35.2	-	63.6	57.3	-
Val			13.1	-	-	25.3	13.9	19.6	65.5	11.4	188.1	253.6	15.1	445.4	528.1	6.2
Met	-	-	-	-	-	-	-	-	-	-	2.1	6.5	-	17.8	25.3	-
Ile	-	-	-	-	-	-	-	-	9.1	-	60.1	98.4	-	166.1	235.9	-
Leu	-	-	-	-	-	-	-	-	24.0	-	100.8	135.6	-	276.9	330.7	3.2
Tyr	-	3.1	5.6	-	7.3	13.1	-	11.0	15.6	-	34.8	41.7	-	65.4	74.9	4.2
Phe	-	-	2.5	-	3.8	7.7	-	6.7	14.2	-	39.2	49.8	-	93.0	113.9	8.6
Orn	-	-	-	-	-	2.7	-	1.5	5.8	-	16.6	21.9	-	42.0	57.0	-
Lys	9.2	29.5	41.1	5.7	58.1	74.0	3.5	73.9	97.7	2.7	173.6	195.9	-	279.4	318.7	-
His	-	6.6	8.6	-	13.0	18.2	-	17.3	21.0	-	35.1	40.2	-	61.3	70.1	-
Pro	-	15.5	41.4	-	56.5	224.1	-	100.4	160.5	-	249.5	293.9	-	387.5	464.0	-

“-“ : not detected

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Table S3. List of oligonucleotides used in this study

MW2 ORF	Gene name	Description of gene product	Forward primer	Reverse primer
MW0005	*gyrB*	gyrase	AATCAGCGTTAGATGTAGCAAGC	GTCACGACCAGATTTTGTAGACC
MW0051	*seh*	enterotoxin H	TGGTCAATATAATCACCCATTCA	TCAAATCATTGCCACTATCACC
MW0100	*butA*	acetoin(diacetyl)reductase	TTATCAATGCAACATCTCAAGCA	CTAAATCTTGTGCGGCTACTTGT
MW0124	*cap8A*	capsular polysaccharide Cap8A	GCGCTATTGTTACATTTTTCGTC	TCTTGTGCCATAAACTGAGGATT
MW0202	*pflA*	formate acetyltransferase enzyme	AGGGACACTTACATTCTGTCGAA	CAAGTATCTGGATTGTGGCAATA
MW0217	*lctE*	Llactate dehydrogenase	AATGAAGATGCCGTATTGACTGT	GATTTCTACGACGTTGCGAATAC
MW0238	*lrgA*	holin-like protein LrgA	CTGTTAAGTTAGGCGAAGTCGAA	GCTTGGCTAATGACACCTAAAGA
MW0477	*ctsR*	transcription repressor of stress genes	CAGCTGATTGGACCTTCTATTTC	GCTTGAATCATTTTAGCTTCACG
MW0480	*clpC*	ATPdependent Clp proteinase chain	AGTTATTGCAGGATGGACAGGTA	TTTGCCCAATAACTCTCTCATGT
MW0516	*sdrC*	SerAsp rich fibrinogenbinding,	GGCATGATACCAAATCGATTAAA	CTTCATGACCACTTAACCCAAAA
MW0568	*adh1*	alcohol dehydrogenase I	TGCTGATTTTGGTGATGTTACAG	TAGACACACGGTCTCCAATTTTT
MW0580	*sarA*	staphylococcal accessory regulator A	TTGCTTTGAGTTGTTATCAATGG	TTTCTCTTTGTTTTCGCTGATGT
MW0604	*pbp4*	penicillin binding protein 4	ATCCAGCGTCTATGACCAAATTA	TTCTTTGTTCGTCATTGTGACAG
MW0648	*mgrA*	transcriptional regulator	CGAAGTCGATCAACGTGAAGTAT	AGAAGAAGCTGAAGCGACTTTGT
MW0667	*saeS*	histidine protein kinase	TCAATATTGCAACCATATGAGCA	AGTGGCGTTCGATATTGATAAAA
MW0705	*pepT*	aminotripeptidase	GATATTCCAGCAAACACTGAACC	GAAGTTACCGCAACCTGTAAAGA
MW0730	*clpP*	ATPdependent Clp protease	GCTGGTTTTGCGATTTATGATAC	TTGCACCAGCTGCTAATAAGAAT
MW0759	*sec4*	enterotoxin C	AAACATGAAGGAAACCACTTTGA	TTTGCACTTCAAAAGAAATTGTG
MW0760	*sel2*	enterotoxin L	GGTTACCGCACAAGAAATAGATG	TGCCGTATTCTTTACCTTTACCA
MW0764	*clfA*	clumping factor A	TTACGAATCAGTTGACGAATGTG	AGGCACTGAAAAACCATAATTCA
MW1028	*trxA*	thioredoxin	TAAAATGATCGCTCCGGTATTAG	TTTAGCTGCAGTTGATGGATTTT
MW1069	*ftsZ*	cell division protein	CTTCAAATTCATCAAATGCACAA	CGTCTTGTTCTTCTTGAACGTCT
MW1129	*sucD*	succinylCoA synthetase	ATGCCTGGCTATATTCACAAAAA	CCAACAGCTGTAGTTTGACCAAT
MW1138	*codY*	transcription pleiotropic repressor	ATGGAAATCTTACGTGAGAAGCA	ATGTTCAATCGCTTCTTTTTCAG
MW1221	*katA*	catalase	ATTTACGTCATATGCATGGGTTC	TTTTCAATACCTTGTTGCGTTCT
MW1237	*citb*	aconitate hydratase	GCGAGGTACCATTTAAACCTTCT	AGTAATATCTCCCCCAACGTCAT
MW1290	*cspA*	cold shock protein A	TCGAAGTTGAAGGAGAAAATGAC	ACTTCAAACTCAACAGCTTGACC
MW1446	*srrA*	staphylococcal respiratory response	ATCTTTTGAAATCCATGAAGCAA	TCCATTTCAGGCAACATTAAATC
MW1505	*sodA*	superoxide dismutase	TTCTGGGAGTTACTTTCACCAAA	CTGCTTTGTCAGCAAATTCTTTT
MW1532	*dnaK*	DnaK protein	AAGGTTCACGTACAACACCATCT	GATTGAACAGTGTTTGGGTTTGT
MW1584	*relA*	GTP pyrophosphokinase	TCGAAGGCGATATAAATGGTAGA	ATAACACGTATCGCCAACAAATC
MW1641	*pykA*	pyruvate kinase	GTATTGGACGTGGATCAGTTGTT	ACAAACGTTTCATCGATTGAGTT
MW1694		XaaHis dipeptidase homolog	AATTATTGAAAAAGGCGACATGA	TCAGTTTAGGATCTGGTTCTCCA
MW1705	*rot*	transcriptional regulator	ATTGGGAGATGTTTAGCATGAAA	TTCAATCTCGCTGAAAATTGAGT
MW1885	*sak*	staphylokinase	CGCAAAGATCGAAGTCACTTATT	ATGCTCTGATAAATCTGGGACAA
MW1889	*sea*	enterotoxin A	TAATCGATTGACCGAAGAGAAAA	ATAACGTCTTGCTTGAAGATCCA
MW1937	*seg2*	enterotoxin G	TACGATTTGTTTTACACCGGAAC	TCCAAATGAAAATTCTCTGCATC
MW1938	*sek2*	enterotoxin K	CTACACAGGAGATGATGGGCTAC	CATCCAAATGGAATTTCTCAGAC
MW1959	*hld (RNAIII)*	deltahemolysin	TAAGGAAGGAGTGATTTCAATGG	GTGAATTTGTTCACTGTGTCGAT
MW1963	*agrA (RNAII)*	accessory gene regulator A	CCTCGCAACTGATAATCCTTATG	ACGAATTTCACTGCCTAATTTGA
MW1977	*ilvD*	dihydroxy-acid dehydratase	GATGAAGAAAGATGGCACGTTAC	GAGGGTGAATGACATCAAAGTTC
MW1981	*leuA*	2-isopropylmalate synthase	CGCTTCTTTATCAATGCCATAAC	AACTTGTTGGTGTAAGCACGACT
MW1988	*sigB*	sigma factor B	TTCACCTGAGCAAATTAACCAAT	ATCTTCGTGATGTGATTGTCCTT
MW2108	*asp23*	alkaline shock protein 23	AGACATGAAAGGTGGCTTAACTG	GCTTGTTTTTCACCAACTTCAAC
MW2115	*lacG*	6phosphobetagalactosidase	AGTATTCTCGTGAAACGATGGAA	GCTGCATCTAAAATGGCATAATC
MW2132	*alsS*	alpha-acetolactate synthase	CCAATTTACCCGTTGTAGAAACA	ATTCATCACCAACTTGATTACGG
MW2212	*ureD*	urease accessory protein	ATTCGATTGTCGTGTTGCTATTT	TCTGCGATATAGCTTTGGATGAT
MW2460	*cidC*	pyruvate oxydase	ACAATGCAGGATGCAGATTTACT	TTTTAGGATTTGTGTCGATTTGG
MW2462	*cidA*	holin-like protein cidA	TGTACCGCTAACTTGGGTAGAAG	TTCGGAAGCAACATCCATAATAC
MW2469	*clpL*	ATPdependent Clp proteinase chain	AAGATGCACTGATTCGACTTGAT	TGTCATCATAACCGACATAACCA
MW2482	*crtN*	staphyloxanthin synthase	AAGATGTTTTTACAGCGTGTGGT	AATCTGTAGGCACCGTTATACGA
MW2526	*mqo2*	malate/quinone oxidoreductase	CGATGCCAAAGTTTATGGTAAAG	AGCAAATGGTCCAAATAACAATG
MW2552	*arcR*	deaminase pathway repressor	TTCTTGGATTGCCTAGAGAATTG	TTCATGTGTTGCTGCTCATTATC
MW2553	*arcC*	carbamate kinase	AATCCAACAAGCTAAATCGAACA	GATTGATTTCAGTTTCCAACCAA
MW2559	*isaB*	immunodominant antigen B	AGGAAGCTGATAGCGGTATTTTT	TGTCCAACTGTTACTTCGACAAC
MW2585	*icaR*	operon ica repressor	AAATCGAACTATTCAATTGATGC	CAGAAAATTCCTCAGGCGTATTA
MW2586	*icaA*	intercellular adhesion protein A	AAGAAGCCTGACATAAATGTGGA	TCTCGTATTTGAGTGCAAGAACA
MW2623	*cspB*	cold shock protein B	AATGGTTTAACGCAGAAAAAGGT	TCTTCTAAAGTTTTGTAGCCATCG