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1 TITLE **Defence against antimicrobial peptides: different strategies in Firmicutes**

2

3 AUTHORS Ainhoa Revilla-Guarinos ¹, Susanne Gebhard ², Thorsten Mascher ², Manuel
4 Zúñiga¹ *

5

6 ¹ Departamento de Biotecnología, Instituto de Agroquímica y Tecnología de Alimentos
7 (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Avda. Agustín Escardino
8 7, 46980 Paterna, Valencia, Spain

9 ² Ludwig-Maximilians-Universität München, Department Biologie I, Mikrobiologie,
10 Großhaderner Str. 2-4, D-82152, Planegg-Martinsried, Germany

11

12 * Corresponding author; Tel +34 963900022; Fax +34 963636301

13 E-mail: btzman@iata.csic.es

14

15 RUNNING TITLE Antimicrobial peptide resistance in Firmicutes

16

17 **Summary**

18 The Firmicutes constitute a phylum of bacteria that can be found in a wide variety of habitats,
19 from soil to the gastrointestinal tract of animals, where they have to thrive in complex
20 communities. Competition in these communities usually involves the production of
21 compounds such as antimicrobial peptides to eliminate competitor organisms. Animals and
22 plants also produce antimicrobial peptides to control their associated microbiota. In turn,
23 defence mechanisms have evolved to prevent the action of these compounds. The close
24 association of some Firmicutes with humans as prominent pathogens or commensal organisms
25 has driven a considerable research effort on defence mechanisms used by these bacteria
26 against antimicrobial compounds. This review focuses on the most recent advances on two
27 well characterized defence mechanisms against antimicrobial peptides: the modification of the
28 cell wall by D-alanylation and the role of peptide antibiotic-specific ABC transporters.

29

30 **Introduction**

31 Antimicrobial peptides (AMPs) are a diverse group of compounds produced by bacteria as well
32 as higher organisms, including animals and humans. Among the most prominent examples of
33 bacterial AMPs are the heavily modified lantibiotics, a class of bacteriocins that were named
34 after their characteristic lanthionine or methyllanthionine residues. Their structure can be
35 either elongated, for example in nisin (Fig. 1A) or subtilin, or globular, as is the case for
36 mersacidin or actagardine (Bierbaum and Sahl, 2009). Non-lantibiotic bacteriocins are similar
37 in size to lantibiotics (<10 kDa), but are not as extensively modified (Cotter et al., 2005). Both
38 classes of bacteriocins are ribosomally synthesized and mainly produced by Firmicutes
39 bacteria. Gram-negative bacteria also produce AMPs, usually referred to as microcins. These
40 are ribosomally synthesized, essentially hydrophobic peptides that in some cases are subjected
41 to post-translational modifications (Rebuffat, 2012). In addition, many bacteria also produce
42 non-ribosomally synthesized peptides such as the small circular metallo-peptide bacitracin (Fig.

43 1A) (Johnson et al., 1945; Economou et al., 2013), or lipodepsipeptides such as ramoplanin
44 (Fig. 1A) or enduracidin (Fang et al., 2006). AMPs also comprise a component of the innate
45 immune system of higher organisms such as protegrins (Fig. 1A). Another example of
46 mammalian AMPs are the defensins, which are produced for example in epithelial and immune
47 cells of humans. They are about 30-40 amino acids long cysteine-rich peptides and adopt a
48 conformation stabilized by disulfide bridges (Yount and Yeaman, 2013). With the exception of
49 microcins, the examples mentioned above share a predominant positive charge and are
50 therefore also referred to as cationic AMPs (CAMPs). Many more examples exist and have
51 been extensively reviewed elsewhere (see for example (Breukink and de Kruijff, 2006; Nguyen
52 et al., 2011; Yount and Yeaman, 2013).

53 AMPs inhibit bacterial growth, either to provide a competitive advantage to the producer in
54 mixed bacterial populations or as a host defence mechanism against pathogens of higher
55 organisms. To combat this, the targeted bacteria have to be able to detect and respond to
56 AMPs in their environment. The sensitivity and efficiency of these processes are important
57 factors for the survival of bacteria in competitive habitats such as the soil or the intestinal tract
58 of mammals.

59 The primary mode-of-action of the AMPs addressed in this review is the inhibition of cell wall
60 synthesis (Fig. 1B), although additional activities have been described for some compounds,
61 e.g. pore-formation by nisin-type lantibiotics (Schneider and Sahl, 2010; Scherer et al., 2013) or
62 disturbance of membrane function by bacitracin (Ming and Epperson, 2002; Schneider and
63 Sahl, 2010; Economou et al., 2013). Details of the synthesis of the peptidoglycan polymer that
64 constitutes the bacterial cell wall have been reviewed elsewhere (van Heijenoort, 2007;
65 Bouhss et al., 2008). In brief, the biosynthetic cycle is initiated on the cytoplasmic side by
66 assembly of precursor molecules and their attachment to the lipid carrier undecaprenyl-
67 phosphate (UP; Fig. 1B). The resulting complex of N-acetylglucosamine-N-acetylmuramyl-
68 pentapeptide, covalently coupled to the lipid carrier via a pyrophosphate linker is referred to

69 as lipid II (Fig. 1B) (van Heijenoort, 2007). After flipping of lipid II to the outer face of the
70 cytoplasmic membrane (Mohammadi et al., 2011), the peptidoglycan subunits are
71 incorporated into the growing cell wall. This step is the target for many CAMPs (Fig. 1B), e.g.
72 the lantibiotics, which bind to the pyrophosphate moiety of lipid II on the outer face of the
73 membrane (Bonev et al., 2004; Hsu et al., 2004).

74 After removal of the peptidoglycan precursors, the lipid carrier remains in the pyrophosphate
75 form (UPP), which is dephosphorylated by UPP-phosphatases (Bouhss et al., 2008) and flipped
76 back to the cytoplasmic face of the membrane (Fig. 1B). This recycling step is inhibited by
77 bacitracin, which tightly binds to the pyrophosphate group and thus prevents the
78 dephosphorylation reaction (Fig. 1B) (Siewert and Strominger, 1967; Storm and Strominger,
79 1973; Schneider and Sahl, 2010; Economou et al., 2013). CAMPs therefore appear to act as
80 competitive inhibitors of cell wall synthetic enzymes, which is in contrast to the irreversible
81 inactivation of penicillin binding proteins by the paradigmatic cell wall-active β -lactam
82 antibiotics (Fisher et al., 2005) and is an important factor to consider when studying CAMP
83 resistance.

84 To counteract CAMP action, bacteria have developed a broad range of resistance mechanisms,
85 which include drug-specific responses such as proteolytic degradation (Sun et al., 2009) or
86 increased production of the inhibited enzyme (Cao and Helmann, 2002), as well as less specific
87 strategies such as biofilm formation (Otto, 2006). This review will concentrate on two major
88 and widely distributed resistance mechanisms employed by Firmicutes to counteract CAMPs
89 that have recently gained a significant amount of attention: changes in cell surface charge and
90 AMP detoxification by transporters.

91 One way of relieving inhibition by CAMPs competing for substrate binding is to reduce the
92 access of the peptides to the surface of the cytoplasmic membrane, i.e. the location of its
93 target molecules. The best understood mechanisms to achieve this are the D-alanylation of
94 teichoic acids, catalyzed by the DltABCD system (Perego et al., 1995; Neuhaus and Baddiley,

95 2003; McBride and Sonenshein, 2011; Reichmann et al., 2013), and the lysinylation of
96 membrane phospholipids by MprF (Peschel et al., 2001; Oku et al., 2004; Andrä et al., 2011).
97 For example, the sensitivity of a *dltA* mutant strain of *Lactobacillus casei* BL23 increased 12.5-
98 fold for nisin, 4.25-fold for vancomycin, 16-fold for plectasin, 4-fold for mersacidin and 2.5-fold
99 for subtilin, relative to the wild type strain (Revilla-Guarinos et al., 2013). In turn, inactivation
100 of MprF in *Staphylococcus aureus* Sa113 resulted in a 28-fold increased sensitivity for nisin, 7-
101 fold for gallidermin or 12-fold for protegrin 3 (Peschel et al., 2001). Both mechanisms are
102 thought to reduce the net negative charge of the cell envelope, thus decreasing electrostatic
103 interactions between CAMPs and the cell (Fig. 2B). Recently, a second mode of action of the
104 Dlt-system was proposed, based on steric hindrance of CAMP passage through the cell wall
105 due to an increased density of the peptidoglycan sacculus (Fig. 2C) (Saar-Dover et al., 2012).
106 A further mechanism of CAMP resistance is by antibiotic-specific ATP-binding cassette (ABC)
107 transporters (Fig. 2), which are thought to remove the peptides from their site of action. A
108 number of these transporters have been described. For example, the BceAB system of *Bacillus*
109 *subtilis* confers resistance to bacitracin. A 143-fold increased sensitivity in *B. subtilis* 168 BceAB
110 defective mutants has been reported (Ohki et al., 2003). Inactivation of the homologous
111 system ABC 09 of *L. casei* BL23 resulted in an increased sensitivity to bacitracin (2-fold), nisin
112 (1.7-fold) plectasin (2-fold) and subtilin (2.5-fold) relative to the wild type strain (Revilla-
113 Guarinos et al., 2013). However, in contrast to canonical drug efflux systems for antibiotics
114 that target intracellular structures, it is less obvious to envision how a transporter could impart
115 efficient resistance against a drug that binds molecules located on the surface of the cell. For
116 some CAMP transporters, a mechanism akin to the “hydrophobic vacuum cleaner” model has
117 been proposed involving translocation of the peptide from the membrane to the culture
118 supernatant (Stein et al., 2003; Stein et al., 2005; Okuda et al., 2008). The different types of
119 CAMP transporters and their proposed functions are covered in detail below.

120

121 **CAMP resistance by D-alanylation of teichoic acids: electrostatic or steric hindrance?**

122 The cell wall of Gram-positive bacteria essentially consists of several layers of peptidoglycan
123 interwoven with additional glycopolymers such as teichoic acids (Neuhaus and Baddiley, 2003).
124 Structure and function of teichoic acids has been the subject of a number of excellent reviews
125 (Neuhaus and Baddiley, 2003; Weidenmaier and Peschel, 2008; Silhavy et al., 2010; Swoboda
126 et al., 2010), and they will not be discussed in detail. Briefly, TAs are linear polymers typically
127 constituted by monomers of glycerol-P or ribitol-P linked by phosphodiester bonds. These
128 polymers can be attached to the peptidoglycan (wall teichoic acids, WTAs) by a glycosidic
129 bridge or to the cell membrane (lipoteichoic acids, LTAs) via a glycolipid anchor (Fig. 2A)
130 (Neuhaus and Baddiley, 2003). To this backbone, a number of substituents can be linked,
131 among them D-alanine, which can be coupled by an ester bond to free hydroxyl groups of the
132 TA backbone or in some cases to glycosidic substituents (Wicken and Baddiley, 1963;
133 Sadovskaya et al., 2004; Sánchez Carballo et al., 2010). However, it must be noted that D-
134 alanylation is not a general characteristic of TAs, and it is apparently limited to Firmicutes
135 (Neuhaus and Baddiley, 2003). The degree of D-alanylation is highly variable and depends on
136 strain background and growth conditions (Perego et al., 1995; Neuhaus and Baddiley, 2003;
137 McCormick et al., 2011).

138 The synthesis of D-alanyl-LTAs is accomplished by the concerted action of four proteins
139 encoded by the *dltABCD* operon (Perego et al., 1995; Neuhaus et al., 1996). DltA catalyzes the
140 synthesis of D-alanyl-AMP from D-alanine and ATP and subsequently transfers this
141 intermediary compound to the D-alanyl carrier protein DltC ((Neuhaus and Baddiley, 2003) and
142 references therein). The role of proteins DltB and DltC remain to be determined. DltB is
143 predicted to possess 12 membrane-spanning domains (Neuhaus et al., 1996) and the
144 hydropathy profile also predicts that DltD is anchored to the cell membrane by an N-terminal
145 hydrophobic domain (Debabov et al., 2000).

146 The regulation of *dlt* operon expression is operated by different mechanisms in different
147 species, and usually it is subject to the control of several regulatory systems within the same
148 organism. In *Bacillus subtilis*, *dlt* is part of the regulons of the extracytoplasmic-function sigma
149 factors σ^X (Cao and Helmann, 2004; Kingston et al., 2013), σ^Y (Guariglia-Oropeza and Helmann,
150 2011) and the two-component system (TCS) YxdJK (Joseph et al., 2004). In staphylococci, the
151 *dlt* operon is positively regulated by the TCS GraRS (*Staphylococcus aureus*; (Li et al., 2007b)) or
152 its homolog ApsRS (*Staphylococcus epidermidis*; (Li et al., 2007a)) in response to CAMPs, and it
153 is repressed by the TCS ArlRS in response to high extracellular concentrations of Mg^{2+} , Ca^{2+} or
154 Na^+ (Koprivnjak et al., 2006). Furthermore, there is evidence indicating that the global
155 regulators Agr (Dunman et al., 2001) and Rot (Saïd-Salim et al., 2003) are also involved in *dlt*
156 regulation in *S. aureus*. In *Lactobacillus casei*, TCS12 regulates the expression of *dlt*, but
157 induction of *dlt* expression in response to nisin was observed in TCS12-defective mutants,
158 indicating that additional regulatory mechanisms also operate in this organism (Revilla-
159 Guarinos et al., 2013).

160 Studies of *dlt* mutants have shown that D-alanylation of TAs has a wide range of physiological
161 consequences in different bacteria as well as in their interactions with other organisms
162 (Neuhaus and Baddiley, 2003; Weidenmaier and Peschel, 2008; Swoboda et al., 2010). This
163 review will only focus on the important role of D-alanylation for the resistance against CAMPs
164 (Fig. 2), as documented by numerous studies (Davie and Brock, 1966; Peschel et al., 1999; Boyd
165 et al., 2000; Abachin et al., 2002; Poyart et al., 2003; Kristian et al., 2005; Fabretti et al., 2006;
166 Kovács et al., 2006; Saar-Dover et al., 2012; Revilla-Guarinos et al., 2013). These observations
167 have been explained by postulating that D-alanylation of TAs would diminish the electrostatic
168 attraction between CAMPs and the cell envelope by reducing the net charge of the cell wall
169 (Fig. 2B) (Peschel et al., 1999; Neuhaus and Baddiley, 2003; Peschel and Sahl, 2006; Swoboda
170 et al., 2010; Anaya-López et al., 2013). This model is in accordance with different experimental
171 observations demonstrating that a lack of alanylation leads to increased binding of several

172 positively charged molecules such as Mg^{2+} (Heptinstall et al., 1970) or cytochrome *c* (Cyt *c*)
173 (Wecke et al., 1997; Peschel et al., 1999; Kristian et al., 2005; Saar-Dover et al., 2012; Revilla-
174 Guarinos et al., 2013) and also the CAMPs gallidermin (Peschel et al., 1999) and vancomycin
175 (Peschel et al., 2000).

176 While this model (Fig. 2B) is generally accepted, a number of recent observations have
177 challenged it. A *dltA* mutant of *Streptococcus agalactiae* was shown to bind three times more
178 Cyt *c* than the wild-type strain. However, no significant differences in binding of a number of
179 CAMPs were detected, indicating that different interactions account for the binding of Cyt *c*
180 and the binding of CAMPs (Saar-Dover et al., 2012). A direct estimation of the net electric
181 charge of *Lactococcus lactis* cells by electrophoretic mobility measurements detected no
182 significant difference in global cell charge between the wild-type strain and a *dltD*-defective
183 mutant (Giaouris et al., 2008). This observation is in accordance with results from similar
184 experiments on *L. casei* in our own laboratory (unpublished results). The estimation of cell
185 electric charge by binding assays relies on the assumption that the interaction between the cell
186 envelope and the ligand is essentially electrostatic and independent of the nature of the
187 ligand. However, the contribution of other interactions should be taken into account. For
188 example, hydrophobic interactions between Cyt *c* and cell membrane lipids have been
189 observed earlier (Rytömaa et al., 1992; Cortese et al., 1995) and might influence the binding
190 affinity for Cyt *c* of the bacterial cell envelope.

191 Based on these and the following observations, an alternative model (Fig. 2C) was recently
192 proposed that suggests that D-alanylation of TAs leads to structural modifications of the cell
193 wall making it more compact and less permeable and hence restricting the access of CAMPs to
194 the membrane (Saar-Dover et al., 2012). In support, these authors showed that the cell wall of
195 a *S. agalactiae dltA* mutant is less dense and its surface is less rigid than that of the wild-type
196 strain. It was shown that binding of CAMPs to LTA was not significantly different between the
197 two strains; however, access of CAMPs to the membrane was increased in the *dltA*-defective

198 mutant. Furthermore, the authors observed that high NaCl concentration reduced the
199 penetration of CAMPs through the cell wall of the *dltA* strain to restore wild-type behaviour.
200 Previous studies had already noted alterations in the cell wall structure in response to the
201 extent of D-alanylation of TAs. Ou and Marquis observed that removal of D-alanyl esters from
202 TAs of *S. aureus* caused an expansion of the cell wall (Ou and Marquis, 1970). Furthermore, it is
203 well established that TAs play a major role in the structure of the cell wall and that the ionic
204 environment is a determinant in the structural transitions of TAs (Doyle et al., 1974; Pal et al.,
205 1990). Incorporation of D-alanyl residues in TAs would change the ionic environment around
206 TAs, thus modulating the conformational transitions of TAs (Neuhaus and Baddiley, 2003; Saar-
207 Dover et al., 2012). These transitions could account for the structural differences observed
208 between the cell walls of D-alanyl-TAs deficient strains and those of the parental strains. Taken
209 together, this evidence supports the idea that D-alanylation of teichoic acids modifies the
210 electrostatic interactions between TAs leading to a strengthening of the cell wall and an
211 increase of its barrier properties (Fig. 2C). This would impede the access of the usually
212 amphipathic CAMPs to the membrane.

213

214 **CAMP resistance by ABC transporter-mediated antibiotic removal**

215 Recently, a classification scheme for ABC transporters involved in the removal of CAMPs from
216 the cell membrane of Firmicutes based on their predicted domain architectures has been
217 proposed (Gebhard, 2012), which was in accordance with functional characteristics such as
218 transport mechanism and regulation. ABC transporters were classified into five groups, each
219 named after one well-characterized example as SunT-type, NisT-type, LanFEG-type, BceAB-
220 type and BcrAB-type transporters. The first two groups are involved in the export of newly
221 synthesized CAMPs and will not be considered further here. The other three groups of
222 transporters will be described in the following section, highlighting the most striking
223 mechanistic aspects of AMP resistance of each group, and a summary of their main

224 characteristics is presented in Table 1. For reasons of conciseness, only some supporting
225 relevant examples will be discussed. Readers are referred to a recent comprehensive review
226 for further information (Gebhard, 2012).

227 Among the resistance transporters two mechanisms of CAMP detoxification can be
228 distinguished (Fig. 3). For LanFEG and BcrAB-type transporters, the transporter is sufficient for
229 partial resistance but additional proteins help to provide full protection from AMPs. In the case
230 of the BceAB-group, the transporter plays a role in sensing, signaling and detoxification of the
231 AMPs.

232

233 **LanFEG and BcrAB type transporters: playing with partners for higher resistance**

234 Most LanFEG-type transporters are involved in self-protection in lantibiotic producer strains,
235 and recognize only a narrow range of related substrates (Otto et al., 1998; Stein et al., 2003;
236 Gebhard, 2012). BcrAB transporters mediate resistance against bacitracin (Podlesek et al.,
237 1995; Neumüller et al., 2001). LanFEG and BcrAB transporters are composed of two permease
238 subunits with six predicted transmembrane helices, which can be encoded by two separate
239 genes (*lanE* and *lanG* in LanFEG-type) or a single gene (*bcrB* in BcrAB-type). The ATPase
240 subunits are encoded by separate genes in both types of transporters (*lanF* and *bcrA*,
241 respectively) (Gebhard, 2012). Phylogenetic analyses have shown that BcrAB and LanFEG are
242 closely related, and they also share functional characteristics (Gebhard, 2012). Several studies
243 reported that these transporters remove lantibiotics from the cytoplasmic membrane and
244 discharge them to the extracellular medium (Stein et al., 2003; Stein et al., 2005; Okuda et al.,
245 2008) (Fig. 3A, step 3). It remains unclear, however, how cells prevent CAMPs from binding
246 again to the cytoplasmic membrane. In this regard, the high degree of co-occurrence of
247 LanFEG-type transporters with LanI or LanH immunity proteins (78%), and of BcrAB-type
248 transporters with UppP (undecaprenyl pyrophosphate phosphatase)-encoding genes (77%)
249 should be noted (Gebhard, 2012). To date, conflicting data is reported on whether

250 transporters and immunity proteins act cooperatively or independently of each other to confer
251 resistance. An independent action has been proposed for the nisin resistance system of
252 *Lactococcus lactis*, constituted by the immunity protein NisI and the transporter NisFEG and
253 for the SpaI-SpaFEG system of *Bacillus subtilis*, which provides self-protection against subtilin
254 (Stein et al., 2003; Stein et al., 2005). Other studies suggested cooperativity between NisI and
255 NisFEG (Ra et al., 1999; Takala et al., 2004; Takala and Saris, 2006), or between the nukacin
256 ISK-1 immunity protein NukH and NukFEG (Okuda et al., 2008). It is attractive to postulate a
257 concerted action of transporters and immunity proteins, which might explain the mechanism
258 of resistance: the transporter would remove cell membrane-associated CAMPs and release
259 them to the external media while immunity proteins would bind and sequester the CAMPs,
260 thus avoiding re-association with the bacterial surface (Fig. 3A, steps 1 and 2) (Takala et al.,
261 2004).

262 BcrAB-type transporters are often encoded in an operon with a UppP encoding gene (Gebhard,
263 2012). It is therefore likely that the bacitracin resistance mechanism of the transporter is
264 tightly linked to UppP activity (Fig. 3A, steps 3 and 4). In fact, it has been shown that increasing
265 UppP activity confers increased resistance to bacitracin (Bernard et al., 2005; Shaaly et al.,
266 2013), whereas its inactivation led to increased sensitivity (Cao and Helmann, 2002; Shaaly et
267 al., 2013). Therefore, maximal protection is most likely ensured when transporter and UppP
268 act concertedly (Podlesek et al., 1995).

269 The efflux mechanism used by these transporters still awaits elucidation although the
270 hydrophobic vacuum-cleaner model, originally proposed for the eukaryotic P-glycoprotein, a
271 multidrug ABC transporter (Raviv et al., 1990), currently receives major acceptance. This model
272 hypothesizes that the target compounds enter the transporter binding sites directly from the
273 membrane and are released to the extracellular medium. Subsequent studies demonstrated
274 that the P-glycoprotein binds its substrates within the inner leaflet of the membrane and
275 releases them to the extracellular medium (Shapiro et al., 1997; Shapiro and Ling, 1998) as

276 postulated by the hydrophobic vacuum cleaner model. In the same way, transport from the
277 inner leaflet to the extracellular medium was demonstrated for the *L. lactis* multidrug
278 resistance ABC transporter LmrA (Bolhuis et al., 1996). However, it remains to be seen if such a
279 mechanism is directly applicable to the CAMP transporters discussed here, whose substrates
280 are most likely located in the outer leaflet of the membrane.

281

282 **BceAB-type transporters: sensors, triggers and detoxification pumps with a broad range of**
283 **substrates**

284 BceAB-type transporters mediate resistance to CAMPs but are usually not associated with
285 biosynthetic loci. In contrast to BcrAB and most LanFEG-type transporters, BceAB-type
286 transporters display a broader substrate range but can also distinguish between structurally
287 similar substrates (Table 1) (Gebhard and Mascher, 2011; Gebhard, 2012). For example, the *B.*
288 *subtilis* PsdAB transporter is able to transport the lantibiotic actagardine but not the similar
289 one mersacidin. At the same time, PsdAB also transports the lipodepsipeptide enduracidin but
290 not the structurally similar ramoplanin (Staroń et al., 2011). The molecular mechanism behind
291 this characteristic is still unclear.

292 The most noticeable feature of BceAB-type transporters is their frequent genetic and
293 functional association with BceRS-type TCS (Fig. 3B) (Joseph et al., 2002; Mascher, 2006;
294 Dintner et al., 2011). A phylogenetic analysis demonstrated the coevolution of these
295 transporters and TCS in Firmicutes, supporting the functional link between them (Dintner et
296 al., 2011). These Bce-like modules, named after the bacitracin resistance module BceRSAB of
297 *Bacillus subtilis* (Mascher et al., 2003; Ohki et al., 2003), are antimicrobial peptide
298 detoxification systems in which the transporter plays a dual role: it mediates AMP
299 resistance/detoxification and is also required for AMP sensing (Rietkötter et al., 2008). The
300 ABC transporter BceAB detects the stimulus, i.e. presence of bacitracin, and transfers the
301 signal to the histidine kinase (HK) BceS, which does not function as a direct sensor but rather

302 as a signal transfer relay to BceR. Activation of the response regulator BceR then induces the
303 expression of *bceAB* and thus ensures resistance. Experimental evidence from a number of
304 homologous systems from *B. subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, and
305 *Lactobacillus casei* has confirmed such a signaling pathway as a general characteristic of Bce-
306 type modules (Rietkötter et al., 2008; Ouyang et al., 2010; Hiron et al., 2011; Staroń et al.,
307 2011; Falord et al., 2012; Revilla-Guarinos et al., 2013). Interestingly, some BceAB-like
308 transporters appear to have developed specified functions. While some display the dual role
309 described above, others function only as a sensor or only as a resistance pump (Fig. 3B). In the
310 latter case, two transporters and one TCS are required to constitute a functional Bce-like
311 module, as will be described in the following paragraphs.

312 Sensing transporters (Fig. 3B, model 5) detect the presence of a CAMP and transfer the signal
313 to their cognate HKs but do not confer resistance. However, it is worth noting that ATP
314 hydrolysis by the transporter is still required for the signaling process (Rietkötter et al., 2008;
315 Hiron et al., 2011). It has been suggested that transport by these transporters takes place at a
316 low rate that is enough for signaling the presence of the antibiotic to the partner HK, but not
317 sufficient for conferring resistance to it (Gebhard and Mascher, 2011). A characteristic feature
318 of Bce-like modules harbouring a sensing ABC is that they usually control an extended regulon
319 that includes ABC transporters (the sensing and/or associated resistance transporters), genes
320 involved in the cell envelope stress response like *dltABCD* and *mprF* and genes for cell wall
321 biosynthesis (Fig. 3B, model 5). In the Aps/GraRS-VraFG system, VraFG is the sensing
322 transporter, and resistance involves expression of the *dlt*-operon and *mprF*, which together
323 with VraFG are under transcriptional control of the TCS Aps/GraRS (Li et al., 2007b; Meehl et
324 al., 2007; Falord et al., 2011; Falord et al., 2012). Another complex Bce-like regulatory network
325 of AMP detoxification modules was recently described in *L. casei* BL23 (Revilla-Guarinos et al.,
326 2013). Module 12 of this strain was shown to be a sensory system controlling CAMPs
327 resistance. ABC12 is the sensory transporter that communicates with TCS12, which in turn

328 ensures the expression of *dltABCD*, *mprF*, and an additional “orphan” BceAB-type ABC
329 transporter that is located in a different position of the chromosome.

330 Dedicated resistance transporters (Fig. 3B, model 6) mediate the actual resistance to the
331 antibiotic, but are not involved in peptide sensing and signaling. They are usually controlled by
332 a not genetically associated BceRS-type TCS, which is typically encoded together with a sensory
333 transporter. A characterized example is the VraDE transporter of *S. aureus*, which mediates
334 resistance to CAMPs and is under control of the BraRSDE module, where BraRS is the TCS and
335 BraDE the sensory transporter (Hiron et al., 2011).

336 The third group of BceAB-type systems consists of ABC transporters with a dual function: they
337 are involved in substrate sensing and signaling and also confer resistance to it. Hence, these
338 transporters regulate their own expression in response to AMPs via BceRS-like TCS (Fig. 3B,
339 model 7) (Rietkötter et al., 2008). Once the inducing compound is removed, the system
340 switches off. An example of these systems is module 09 of *L. casei*. ABC09 mediates resistance
341 to bacitracin, nisin, plectasin, and subtilin (Revilla-Guarinos et al., 2013). Its expression is
342 induced in a concentration dependent manner by nisin through the cognate TCS09, which
343 depends on ABC09 for its activation. Accordingly, module 09 is a stand-alone resistance
344 module where ABC09 senses the target CAMPs and transfer the signal to TCS09, resulting in
345 the induction of the expression of ABC09, which confers the resistance (Revilla-Guarinos et al.,
346 2013). The same is true for at least two out of the three *B. subtilis* Bce-type resistance
347 modules, which also possess transporters with a dual function. BceRSAB is the most effective
348 bacitracin resistance system (Mascher et al., 2003; Ohki et al., 2003), but it also confers
349 resistance to mersacidin, actagardine and plectasin (Staroń et al., 2011). The paralogous
350 system PsdRSAB is induced by enduracidin, actagardine, gallidermin, nisin and subtilin, and it
351 mediates resistance to all of its inducers excepting actagardine (Staroń et al., 2011). Both
352 systems are stand-alone detoxification modules (Ohki et al., 2003; Rietkötter et al., 2008; Staroń
353 et al., 2011).

354 Although the role of Bce-type resistance modules in the regulation of transcription has been
355 thoroughly studied, details of the mechanism of transport and signal transduction have not
356 been completely determined.

357

358 **Open questions and concluding remarks**

359 Significant progress has been made in the last years to understand the major systems that
360 confer CAMP resistance in Firmicutes, both with regard to the role of D-alanylation and the
361 function of designated ABC transporters. Nevertheless, the studies summarized above have
362 also led to a number of open questions that still need to be addressed in order to provide
363 mechanistic insights into how those systems work.

364 The identification of a potential second mechanism by which D-alanylation of TAs affects
365 CAMP sensitivity raises the question, whether electrostatic and steric hindrance are mutually
366 exclusive concepts or whether both contribute to CAMP resistance (Fig. 2). The evidence for
367 both mechanisms argues in favor of the latter, but further studies will be necessary to answer
368 these questions.

369 In the transporter-mediated resistance, both the mechanism of substrate binding and, in the
370 case of BceAB-like systems, the direction of transport have not been determined so far (Table
371 1). BceB-like permeases are membrane proteins with ten predicted transmembrane helices
372 and a large extracytoplasmic domain (ECD) of approximately 200 amino acids. While
373 phylogenetic analyses of the transmembrane regions of BceB-like transport permeases
374 showed good sequence conservation at the amino acid level, the ECD regions did not (Dintner
375 et al., 2011). This high degree of variability of the ECD agrees with the proposal that this region
376 of the permease contains the substrate binding domain of the transporter (Rietkötter et al.,
377 2008), and that the high degree of variability reflects the wide range of CAMPs to which they
378 confer resistance (Dintner et al., 2011). Some experimental results support this idea. The *S.*
379 *aureus* ABC transporter VraDE confers bacitracin resistance while VraFG is involved in

380 resistance to colistin. Domain-swapping studies showed that a transporter with a chimeric
381 VraG permease harbouring the ECD of VraE, *vraFG*^{*vraE}, restored bacitracin resistance in a
382 Δ *vraDE* mutant but was not able to restore colistin resistance in a Δ *vraFG* mutant strain (Hiron
383 et al., 2011).

384 Moreover, the exact molecular mechanism by means of which the signal information is
385 transferred from the transporter to the HK is also not known yet. It has been proposed that
386 BceAB-type transporters might function as importers so that detection by the cognate HKs and
387 CAMP inactivation would occur in the cytoplasm (Rietkötter et al., 2008; Hiron et al., 2011).
388 However, the identification of mutations in BceB that significantly decreased signaling activity
389 while retaining bacitracin resistance seems to rule out this hypothesis, at least for the BceRSAB
390 module (Kallenberg et al., 2013). A second hypothesis postulates that the transporter binds the
391 substrate and presents it to the HK, which would then only recognize it in complex with the
392 transporter (Schrecke et al., 2012). In this case signal detection by the HK might occur through
393 the short extracytoplasmatic loop of the HK. This idea is supported by results obtained with
394 the homologous HKs GraS of *S. aureus* and ApsS of *S. epidermidis*. These two proteins show an
395 overall 70% similarity, which is reduced to 33% for the extracellular loop. ApsS responds to
396 hBD3 whereas GraS does not. However, a hybrid GraS with the ApsS extracellular loop responds
397 to hBD3 (Li et al., 2007b). But this hypothesis does not explain why ATP hydrolysis by the
398 transporter is required for signal transfer, since substrate binding should be ATP-independent.

399 The third hypothesis postulates that signal transfer occurs by direct protein-protein contact
400 between the ABC transporter and the HK, where a conformational change in the transporter
401 due to substrate binding and transport could activate the HK. This hypothesis is supported by
402 results obtained by two-hybrid assays carried out with the GraXSR-VraFG system of *S. aureus*
403 (Falord et al., 2012) and the BceRSAB of *B. subtilis* (Kallenberg et al., 2013) which revealed
404 interactions between HKs and cognate ABC transporters.

405 Different strategies for CAMP resistance in Firmicutes have been reviewed in this work, which,
406 while being very distinct from one another, all serve the same purpose: to enhance bacterial
407 survival in competitive environments. The recently proposed electrostatic-steric hindrance
408 model for the Dlt-mediated resistance as well as the many unanswered questions regarding
409 ABC transporters, highlight the complexity of this subject. Given the significant amount of
410 progress made in recent years and the diversity of different organisms and experimental
411 approaches currently applied to study both mechanisms of CAMP resistance, one can be
412 optimistic that some, if not most of the above questions will be addressed and eventually
413 solved.

414

415 **References**

416

- 417 Abachin, E., Poyart, C., Pellegrini, E., Milohanic, E., Fiedler, F., Berche, P., and Trieu-Cuot, P.
418 (2002) Formation of D-alanyl-lipoteichoic acid is required for adhesion and virulence of *Listeria*
419 *monocytogenes*. *Mol Microbiol* **43**: 1-14.
- 420 Anaya-López, J.L., López-Meza, J.E., and Ochoa-Zarzosa, A. (2013) Bacterial resistance to
421 cationic antimicrobial peptides. *Crit Rev Microbiol* **39**: 180-195.
- 422 Andrä, J., Goldmann, T., Ernst, C.M., Peschel, A., and Gutschmann, T. (2011) Multiple peptide
423 resistance factor (MprF)-mediated resistance of *Staphylococcus aureus* against antimicrobial
424 peptides coincides with a modulated peptide interaction with artificial membranes comprising
425 lysyl-phosphatidylglycerol. *J Biol Chem* **286**: 18692-18700.
- 426 Bernard, R., El Ghachi, M., Mengin-Lecreulx, D., Chippaux, M., and Denizot, F. (2005) BcrC from
427 *Bacillus subtilis* acts as an undecaprenyl pyrophosphate phosphatase in bacitracin resistance. *J*
428 *Biol Chem* **280**: 28852-28857.
- 429 Bierbaum, G., and Sahl, H.G. (2009) Lantibiotics: mode of action, biosynthesis and
430 bioengineering. *Curr Pharm Biotechnol* **10**: 2-18.
- 431 Bolhuis, H., Van Veen, H.W., Molenaar, D., Poolman, B., Driessen, A.J.M., and Konings, W.N.
432 (1996) Multidrug resistance in *Lactococcus lactis*: evidence for ATP-dependent drug extrusion
433 from the inner leaflet of the cytoplasmic membrane. *EMBO J* **15**: 4239-4245.
- 434 Bonev, B.B., Breukink, E., Swiezewska, E., De Kruijff, B., and Watts, A. (2004) Targeting
435 extracellular pyrophosphates underpins the high selectivity of nisin. *FASEB J* **18**: 1862-1869.
- 436 Bouhss, A., Trunkfield, A.E., Bugg, T.D.H., and Mengin-Lecreulx, D. (2008) The biosynthesis of
437 peptidoglycan lipid-linked intermediates. *FEMS Microbiol Rev* **32**: 208-233.
- 438 Boyd, D.A., Cvitkovitch, D.G., Bleiweis, A.S., Kiriukhin, M.Y., Debabov, D.V., Neuhaus, F.C., and
439 Hamilton, I.R. (2000) Defects in D-alanyl-lipoteichoic acid synthesis in *Streptococcus mutans*
440 results in acid sensitivity. *J Bacteriol* **182**: 6055-6065.
- 441 Breukink, E., and de Kruijff, B. (2006) Lipid II as a target for antibiotics. *Nat Rev Drug Discov* **5**:
442 321-332.
- 443 Cao, M., and Helmann, J.D. (2002) Regulation of the *Bacillus subtilis* *bcrC* bacitracin resistance
444 gene by two extracytoplasmic function sigma factors. *J Bacteriol* **184**: 6123-6129.

445 Cao, M., and Helmann, J.D. (2004) The *Bacillus subtilis* extracytoplasmic-function σ^X factor
446 regulates modification of the cell envelope and resistance to cationic antimicrobial peptides. *J*
447 *Bacteriol* **186**: 1136-1146.

448 Cortese, J.D., Voglino, A.L., and Hackenbrock, C.R. (1995) Persistence of cytochrome c binding
449 to membranes at physiological mitochondrial intermembrane space ionic strength. *Biochim*
450 *Biophys Acta* **1228**: 216-228.

451 Cotter, P.D., Hill, C., and Ross, R.P. (2005) Bacteriocins: developing innate immunity for food.
452 *Nat Rev Microbiol* **3**: 777-788.

453 Davie, J.M., and Brock, T.D. (1966) Effect of teichoic acid on resistance to membrane-lytic
454 agent of *Streptococcus zymogenes*. *J Bacteriol* **92**: 1623-&.

455 Debabov, D.V., Kiriukhin, M.Y., and Neuhaus, F.C. (2000) Biosynthesis of lipoteichoic acid in
456 *Lactobacillus rhamnosus*: Role of DltD in D-alanylation. *J Bacteriol* **182**: 2855-2864.

457 Dintner, S., Staron, A., Berchtold, E., Petri, T., Mascher, T., and Gebhard, S. (2011) Coevolution
458 of ABC transporters and two-component regulatory systems as resistance modules against
459 antimicrobial peptides in Firmicutes bacteria. *J Bacteriol* **193**: 3851-3862.

460 Doyle, R.J., McDannel, M.L., Streips, U.N., Birdsell, D.C., and Young, F.E. (1974) Polyelectrolyte
461 nature of bacterial teichoic acids. *J Bacteriol* **118**: 606-615.

462 Dunman, P.M., Murphy, E., Haney, S., Palacios, D., Tucker-Kellogg, G., Wu, S. et al. (2001)
463 Transcription profiling-based identification of *Staphylococcus aureus* genes regulated by the
464 *agr* and/or *sarA* loci. *J Bacteriol* **183**: 7341-7353.

465 Economou, N.J., Cocklin, S., and Loll, P.J. (2013) High-resolution crystal structure reveals
466 molecular details of target recognition by bacitracin. *Proc Natl Acad Sci U S A* **110**: 14207-
467 14212.

468 Fabretti, F., Theilacker, C., Baldassarri, L., Kaczynski, Z., Kropec, A., Holst, O., and Huebner, J.
469 (2006) Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and
470 resistance to antimicrobial peptides. *Infect Immun* **74**: 4164-4171.

471 Falord, M., Karimova, G., Hiron, A., and Msadek, T. (2012) GraXSR proteins interact with the
472 *VraFG* ABC transporter to form a five-component system required for cationic antimicrobial
473 peptide sensing and resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **56**:
474 1047-1058.

475 Falord, M., Mäder, U., Hiron, A., Débarbouillé, M., and Msadek, T. (2011) Investigation of the
476 *Staphylococcus aureus* GraSR regulon reveals novel links to virulence, stress response and cell
477 wall signal transduction pathways. *PLoS One* **6**: e21323.

478 Fang, X., Tiyanont, K., Zhang, Y., Wanner, J., Boger, D., and Walker, S. (2006) The mechanism of
479 action of ramoplanin and enduracidin. *Mol Biosyst* **2**: 69-76.

480 Fisher, J.F., Meroueh, S.O., and Mobashery, S. (2005) Bacterial resistance to β -lactam
481 antibiotics: Compelling opportunism, compelling opportunity. *Chem Rev* **105**: 395-424.

482 Gebhard, S. (2012) ABC transporters of antimicrobial peptides in Firmicutes bacteria:
483 phylogeny, function and regulation. *Mol Microbiol* **86**: 1295-1317.

484 Gebhard, S., and Mascher, T. (2011) Antimicrobial peptide sensing and detoxification modules:
485 unravelling the regulatory circuitry of *Staphylococcus aureus*. *Mol Microbiol* **81**: 581-587.

486 Giaouris, E., Briandet, R., Meyrand, M., Courtin, P., and Chapot-Chartier, M.P. (2008) Variations
487 in the degree of D-alanylation of teichoic acids in *Lactococcus lactis* alter resistance to cationic
488 antimicrobials but have no effect on bacterial surface hydrophobicity and charge. *Appl Environ*
489 *Microbiol* **74**: 4764-4767.

490 Guariglia-Oropeza, V., and Helmann, J.D. (2011) *Bacillus subtilis* σ^V confers lysozyme resistance
491 by activation of two cell wall modification pathways, peptidoglycan O-acetylation and D-
492 alanylation of teichoic acids. *J Bacteriol* **193**: 6223-6232.

493 Heptinstall, S., Archibald, A.R., and Baddiley, J. (1970) Teichoic acids and membrane function in
494 bacteria. *Nature* **225**: 519-521.

495 Hiron, A., Falord, M., Valle, J., Debarbouille, M., and Msadek, T. (2011) Bacitracin and nisin
496 resistance in *Staphylococcus aureus*: a novel pathway involving the BraS/BraR two-component

497 system (SA2417/SA2418) and both the BraD/BraE and VraD/VraE ABC transporters. *Mol*
498 *Microbiol* **81**: 602-622.

499 Hsu, S.T., Breukink, E., Tischenko, E., Lutters, M.A., de Kruijff, B., Kaptein, R. et al. (2004) The
500 nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel
501 antibiotics. *Nat Struct Mol Biol* **11**: 963-967.

502 Johnson, B.A., Anker, H., and Meleney, F.L. (1945) Bacitracin: A new antibiotic produced by a
503 member of the *B. subtilis* group. *Science* **102**: 376-377.

504 Joseph, P., Fichant, G., Quentin, Y., and Denizot, F. (2002) Regulatory relationship of two-
505 component and ABC transport systems and clustering of their genes in the *Bacillus/Clostridium*
506 group, suggest a functional link between them. *J Mol Microbiol Biotechnol* **4**: 503-513.

507 Joseph, P., Guiseppi, A., Sorokin, A., and Denizot, F. (2004) Characterization of the *Bacillus*
508 *subtilis* YxdJ response regulator as the inducer of expression for the cognate ABC transporter
509 YxdLM. *Microbiology* **150**: 2609-2617.

510 Kallenberg, F., Dintner, S., Schmitz, R., and Gebhard, S. (2013) Identification of regions
511 important for resistance and signalling within the antimicrobial peptide transporter BceAB of
512 *Bacillus subtilis*. *J Bacteriol* **195**: 3287-3297.

513 Kingston, A.W., Liao, X., and Helmann, J.D. (2013) Contributions of the σ^W , σ^M , and σ^X
514 regulons to the lantibiotic resistome of *Bacillus subtilis*. *Mol Microbiol*: DOI:
515 10.1111/mmi.12380.

516 Koprivnjak, T., Mlakar, V., Swanson, L., Fournier, B., Peschel, A., and Weiss, J.P. (2006) Cation-
517 induced transcriptional regulation of the *dlt* operon of *Staphylococcus aureus*. *J Bacteriol* **188**:
518 3622-3630.

519 Kovács, M., Halfmann, A., Fedtke, I., Heintz, M., Peschel, A., Vollmer, W. et al. (2006) A
520 functional *dlt* operon, encoding proteins required for incorporation of D-alanine in teichoic
521 acids in gram-positive bacteria, confers resistance to cationic antimicrobial peptides in
522 *Streptococcus pneumoniae*. *J Bacteriol* **188**: 5797-5805.

523 Kristian, S.A., Datta, V., Weidenmaier, C., Kansal, R., Fedtke, I., Peschel, A. et al. (2005) D-
524 alanylation of teichoic acids promotes group A *Streptococcus* antimicrobial peptide resistance,
525 neutrophil survival, and epithelial cell invasion. *J Bacteriol* **187**: 6719-6725.

526 Li, M., Lai, Y., Villaruz, A.E., Cha, D.J., Sturdevant, D.E., and Otto, M. (2007a) Gram-positive
527 three-component antimicrobial peptide-sensing system. *Proc Natl Acad Sci USA* **104**: 9469-
528 9474.

529 Li, M., Cha, D.J., Lai, Y., Villaruz, A.E., Sturdevant, D.E., and Otto, M. (2007b) The antimicrobial
530 peptide-sensing system *aps* of *Staphylococcus aureus*. *Mol Microbiol* **66**: 1136-1147.

531 Mascher, T. (2006) Intramembrane-sensing histidine kinases: a new family of cell envelope
532 stress sensors in Firmicutes bacteria. *FEMS Microbiol Lett* **264**: 133-144.

533 Mascher, T., Margulis, N.G., Wang, T., Ye, R.W., and Helmann, J.D. (2003) Cell wall stress
534 responses in *Bacillus subtilis*: the regulatory network of the bacitracin stimulon. *Mol Microbiol*
535 **50**: 1591-1604.

536 Mascher, T., Heintz, M., Zahner, D., Merai, M., and Hakenbeck, R. (2006) The CiaRH system of
537 *Streptococcus pneumoniae* prevents lysis during stress induced by treatment with cell wall
538 inhibitors and by mutations in *pbp2x* involved in beta-lactam resistance. *J Bacteriol* **188**: 1959-
539 1968.

540 McBride, S.M., and Sonenshein, A.L. (2011) Identification of a genetic locus responsible for
541 antimicrobial peptide resistance in *Clostridium difficile*. *Infect Immun* **79**: 167-176.

542 McCormick, N.E., Halperin, S.A., and Lee, S.F. (2011) Regulation of D-alanylation of lipoteichoic
543 acid in *Streptococcus gordonii*. *Microbiology* **157**: 2248-2256.

544 Meehl, M., Herbert, S., Götz, F., and Cheung, A. (2007) Interaction of the GraRS two-
545 component system with the VraFG ABC transporter to support vancomycin-intermediate
546 resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **51**: 2679-2689.

547 Ming, L.-J., and Epperson, J.D. (2002) Metal binding and structure–activity relationship of the
548 metalloantibiotic peptide bacitracin. *J Inorg Biochem* **91**: 46-58.

549 Mohammadi, T., van Dam, V., Sijbrandi, R., Vernet, T., Zapun, A., Bouhss, A. et al. (2011)
550 Identification of FtsW as a transporter of lipid-linked cell wall precursors across the membrane.
551 *EMBO J* **30**: 1425-1432.

552 Neuhaus, F.C., and Baddiley, J. (2003) A continuum of anionic charge: structures and functions
553 of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiol Mol Biol Rev* **67**: 686-723.

554 Neuhaus, F.C., Heaton, M.P., Debatov, D.V., and Zhang, Q. (1996) The *dlt* operon in the
555 biosynthesis of D-alanyl-lipoteichoic acid in *Lactobacillus casei*. *Microb Drug Resist* **2**: 77-84.

556 Neumüller, A.M., Konz, D., and Marahiel, M.A. (2001) The two-component regulatory system
557 BacRS is associated with bacitracin 'self-resistance' of *Bacillus licheniformis* ATCC 10716. *Eur J*
558 *Biochem* **268**: 3180-3189.

559 Nguyen, L.T., Haney, E.F., and Vogel, H.J. (2011) The expanding scope of antimicrobial peptide
560 structures and their modes of action. *Trends Biotechnol* **29**: 464-472.

561 Ohki, R., Giyanto, Tateno, K., Masuyama, W., Moriya, S., Kobayashi, K., and Ogasawara, N.
562 (2003) The BceRS two-component regulatory system induces expression of the bacitracin
563 transporter, BceAB, in *Bacillus subtilis*. *Mol Microbiol* **49**: 1135-1144.

564 Oku, Y., Kurokawa, K., Ichihashi, N., and Sekimizu, K. (2004) Characterization of the
565 *Staphylococcus aureus mprF* gene, involved in lysinylation of phosphatidylglycerol.
566 *Microbiology* **150**: 45-51.

567 Okuda, K., Aso, Y., Nakayama, J., and Sonomoto, K. (2008) Cooperative transport between
568 NukFEG and NukH in immunity against the lantibiotic nukacin ISK-1 produced by
569 *Staphylococcus warneri* ISK-1. *J Bacteriol* **190**: 356-362.

570 Otto, M. (2006) Bacterial evasion of antimicrobial peptides by biofilm formation. In
571 *Antimicrobial Peptides and Human Disease*. Shafer, W.M. (ed): Springer Berlin Heidelberg, pp.
572 251-258.

573 Otto, M., Peschel, A., and Gotz, F. (1998) Producer self-protection against the lantibiotic
574 epidermin by the ABC transporter EpiFEG of *Staphylococcus epidermidis* Tu3298. *FEMS*
575 *Microbiol Lett* **166**: 203-211.

576 Ou, L.T., and Marquis, R.E. (1970) Electromechanical interactions in cell walls of gram-positive
577 cocci. *J Bacteriol* **101**: 92-101.

578 Ouyang, J., Tian, X.L., Versey, J., Wishart, A., and Li, Y.H. (2010) The BceABRS four-component
579 system regulates the bacitracin-induced cell envelope stress response in *Streptococcus*
580 *mutans*. *Antimicrob Agents Chemother* **54**: 3895-3906.

581 Pal, M.K., Ghosh, T.C., and Ghosh, J.K. (1990) Studies on the conformation of and metal ion
582 binding by teichoic acid of *Staphylococcus aureus*. *Biopolymers* **30**: 273-277.

583 Perego, M., Glaser, P., Minutello, A., Strauch, M.A., Leopold, K., and Fischer, W. (1995)
584 Incorporation of D-alanine into lipoteichoic acid and wall teichoic acid in *Bacillus subtilis*:
585 identification of genes and regulation. *J Biol Chem* **270**: 15598-15606.

586 Peschel, A., and Sahl, H.G. (2006) The co-evolution of host cationic antimicrobial peptides and
587 microbial resistance. *Nat Rev Microbiol* **4**: 529-536.

588 Peschel, A., Vuong, C., Otto, M., and Götz, F. (2000) The D-alanine residues of *Staphylococcus*
589 *aureus* teichoic acids alter the susceptibility to vancomycin and the activity of autolytic
590 enzymes. *Antimicrob Agents Chemother* **44**: 2845-2847.

591 Peschel, A., Otto, M., Jack, R.W., Kalbacher, H., Jung, G., and Götz, F. (1999) Inactivation of the
592 *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other
593 antimicrobial peptides. *J Biol Chem* **274**: 8405-8410.

594 Peschel, A., Jack, R.W., Otto, M., Collins, L.V., Staubitz, P., Nicholson, G. et al. (2001)
595 *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the
596 novel virulence factor MprF is based on modification of membrane lipids with L-lysine. *J Exp*
597 *Med* **193**: 1067-1076.

598 Podlesek, Z., Comino, A., Herzog-Velikonja, B., Žgur-Bertok, D., Komel, R., and Grabnar, M.
599 (1995) *Bacillus licheniformis* bacitracin-resistance ABC transporter: Relationship to mammalian
600 multidrug resistance. *Mol Microbiol* **16**: 969-976.

601 Poyart, C., Pellegrini, E., Marceau, M., Baptista, M., Jaubert, F., Lamy, M.-C., and Trieu-Cuot, P.
602 (2003) Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid
603 is due to an increased susceptibility to defensins and phagocytic cells. *Mol Microbiol* **49**: 1615-
604 1625.

605 Ra, R., Beerthuyzen, M.M., de Vos, W.M., Saris, P.E., and Kuipers, O.P. (1999) Effects of gene
606 disruptions in the nisin gene cluster of *Lactococcus lactis* on nisin production and producer
607 immunity. *Microbiology* **145**: 1227-1233.

608 Raviv, Y., Pollard, H.B., Bruggemann, E.P., Pastan, I., and Gottesman, M.M. (1990)
609 Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor
610 cells. *J Biol Chem* **265**: 3975-3980.

611 Rebuffat, S. (2012) Microcins in action: amazing defence strategies of Enterobacteria.
612 *Biochemical Society Transactions* **40**: 1456-1462.

613 Reichmann, N.T., Cassona, C.P., and Gründling, A. (2013) Revised mechanism of D-alanine
614 incorporation into cell wall polymers in Gram-positive bacteria. *Microbiology* **159**: 1868-1877.

615 Revilla-Guarinos, A., Gebhard, S., Alcántara, C., Staroń, A., Mascher, T., and Zúñiga, M. (2013)
616 Characterization of a regulatory network of peptide antibiotic detoxification modules in
617 *Lactobacillus casei* BL23. *Appl Environ Microbiol* **79**: 3160-3170.

618 Rietkötter, E., Hoyer, D., and Mascher, T. (2008) Bacitracin sensing in *Bacillus subtilis*. *Mol*
619 *Microbiol* **68**: 768-785.

620 Rytömaa, M., Mustonen, P., and Kinnunen, P.K. (1992) Reversible, nonionic, and pH-dependent
621 association of cytochrome *c* with cardiolipin-phosphatidylcholine liposomes. *J Biol Chem* **267**:
622 22243-22248.

623 Saar-Dover, R., Bitler, A., Nezer, R., Shmuel-Galia, L., Firon, A., Shimoni, E. et al. (2012) D-
624 alanylation of lipoteichoic acids confers resistance to cationic peptides in group B
625 *Streptococcus* by increasing the cell wall density. *PLoS Pathog* **8**: e1002891.

626 Sadvovskaya, I., Vinogradov, E., Li, J., and Jabbouri, S.d. (2004) Structural elucidation of the
627 extracellular and cell-wall teichoic acids of *Staphylococcus epidermidis* RP62A, a reference
628 biofilm-positive strain. *Carbohydr Res* **339**: 1467-1473.

629 Saïd-Salim, B., Dunman, P.M., McAleese, F.M., Macapagal, D., Murphy, E., McNamara, P.J. et
630 al. (2003) Global regulation of *Staphylococcus aureus* genes by Rot. *J Bacteriol* **185**: 610-619.

631 Sánchez Carballo, P.M., Vilen, H., Palva, A., and Holst, O. (2010) Structural characterization of
632 teichoic acids from *Lactobacillus brevis*. *Carbohydr Res* **345**: 538-542.

633 Scherer, K., Wiedemann, I., Ciobanasu, C., Sahl, H.-G., and Kubitscheck, U. (2013) Aggregates of
634 nisin with various bactoprenol-containing cell wall precursors differ in size and membrane
635 permeation capacity. *Biochim Biophys Acta (BBA) - Biomembranes* **1828**: 2628-2636.

636 Schneider, T., and Sahl, H.-G. (2010) An oldie but a goodie: cell wall biosynthesis as antibiotic
637 target pathway. *Int J Med Microbiol* **300**: 161-169.

638 Schrecke, K., Staroń, A., and Mascher, T. (2012) Gram-positive envelope stress response:
639 intramembrane-sensing histidine kinases and accessory membrane proteins. In *Two*
640 *component systems in bacteria*. Gross, R., and Beier, D. (eds). Norfolk, UK: Caister Academic
641 Press, pp. 199-229.

642 Shaaly, A., Kalamorz, F., Gebhard, S., and Cook, G.M. (2013) Undecaprenyl pyrophosphate
643 phosphatase confers low-level resistance to bacitracin in *Enterococcus faecalis*. *J Antimicrob*
644 *Chemother* **68**: 1583-1593.

645 Shapiro, A.B., and Ling, V. (1998) Transport of LDS-751 from the cytoplasmic leaflet of the
646 plasma membrane by the rhodamine-123-selective site of P-glycoprotein. *Eur J Biochem* **254**:
647 181-188.

648 Shapiro, A.B., Corder, A.B., and Ling, V. (1997) P-glycoprotein-mediated Hoechst 33342
649 transport out of the lipid bilayer. *Eur J Biochem* **250**: 115-121.

650 Siewert, G., and Strominger, J.L. (1967) Bacitracin: an inhibitor of the dephosphorylation of
651 lipid pyrophosphate, an intermediate in the biosynthesis of the peptidoglycan of bacterial cell
652 walls. *Proc Natl Acad Sci U S A* **57**: 767-773.

653 Silhavy, T.J., Kahne, D., and Walker, S. (2010) The bacterial cell envelope. *Cold Spring Harb*
654 *Perspect Biol* **2**: a000414.

655 Staroń, A., Finkeisen, D.E., and Mascher, T. (2011) Peptide antibiotic sensing and detoxification
656 modules of *Bacillus subtilis*. *Antimicrob Agents Chemother* **55**: 515-525.

657 Stein, T., Heinzmann, S., Solovieva, I., and Entian, K.D. (2003) Function of *Lactococcus lactis*
658 nisin immunity genes *nisl* and *nisFEG* after coordinated expression in the surrogate host
659 *Bacillus subtilis*. *J Biol Chem* **278**: 89-94.

660 Stein, T., Heinzmann, S., Düsterhus, S., Borchert, S., and Entian, K.D. (2005) Expression and
661 functional analysis of the subtilin immunity genes *spaFEG* in the subtilin-sensitive host *Bacillus*
662 *subtilis* MO1099. *J Bacteriol* **187**: 822-828.

663 Storm, D.R., and Strominger, J.L. (1973) Complex formation between bacitracin peptides and
664 isoprenyl pyrophosphates: The specificity of lipid-peptide interactions. *J Biol Chem* **248**: 3940-
665 3945.

666 Sun, Z., Zhong, J., Liang, X., Liu, J., Chen, X., and Huan, L. (2009) Novel mechanism for nisin
667 resistance via proteolytic degradation of nisin by the nisin resistance protein NSR. *Antimicrob*
668 *Agents Chemother* **53**: 1964-1973.

669 Swoboda, J.G., Campbell, J., Meredith, T.C., and Walker, S. (2010) Wall teichoic acid function,
670 biosynthesis, and inhibition. *ChemBioChem* **11**: 35-45.

671 Takala, T.M., and Saris, P.E. (2006) C terminus of Nisl provides specificity to nisin. *Microbiology*
672 **152**: 3543-3549.

673 Takala, T.M., Koponen, O., Qiao, M., and Saris, P.E.J. (2004) Lipid-free Nisl: interaction with
674 nisin and contribution to nisin immunity via secretion. *FEMS Microbiol Lett* **237**: 171-177.

675 van Heijenoort, J. (2007) Lipid intermediates in the biosynthesis of bacterial peptidoglycan.
676 *Microbiol Mol Biol Rev* **71**: 620-635.

677 Wecke, J., Madela, K., and Fischer, W. (1997) The absence of D-alanine from lipoteichoic acid
678 and wall teichoic acid alters surface charge, enhances autolysis and increases susceptibility to
679 methicillin in *Bacillus subtilis*. *Microbiology* **143**: 2953-2960.

680 Weidenmaier, C., and Peschel, A. (2008) Teichoic acids and related cell-wall glycopolymers in
681 Gram-positive physiology and host interactions. *Nat Rev Micro* **6**: 276-287.

682 Wicken, A.J., and Baddiley, J. (1963) Structure of intracellular teichoic acids from group D
683 streptococci. *Biochem J* **87**: 54-62.

684 Yount, N.Y., and Yeaman, M.R. (2013) Peptide antimicrobials: cell wall as a bacterial target.
685 *Ann N Y Acad Sci* **1277**: 127-138.

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687

688 **Figure legends**

689 **Fig. 1. A. Structural and compositional diversity of antimicrobial peptides.** Schematic
690 representations of the structures of nisin, bacitracin, ramoplanin and protegrin. The amino
691 acids are represented by labeled gray circles. Positively and negatively charged amino acids at
692 neutral pH are highlighted in red and white, respectively. Abu, aminobutyric acid; Chp, L-3-
693 chloro-4-hydroxyphenylglycine; Dha, didehydroalanine; Dhb, didehydrobutyrine; HAsn, β -
694 hydroxyasparagine; D-Hpg, D-hydroxyphenylglycine; L-Hpg, L-hydroxyphenylglycine; Man,
695 mannose; Orn, D-ornithine; aThr, D-*allo*-threonine.

696 **B. Schematic representation of peptidoglycan biosynthesis and its inhibition by CAMPs.**

697 Important steps in cell wall biosynthesis are depicted, and their cellular location is indicated
698 on the left. CW, cell wall; CM, cytoplasmic membrane; NAG, N-acetyl-glucosamine; NAM,
699 N-acetyl-muramic acid; UP, undecaprenyl-phosphate; UPP, undecaprenyl-pyrophosphate.
700 Amino acids are symbolized by small grey circles. Lipid II consists of the NAG/NAM-
701 pentapeptide building block, covalently linked to the lipid carrier molecule UP via a
702 pyrophosphate ester bridge. The steps of cell envelope biosynthesis linked to UP are referred
703 to as "Lipid II cycle". CAMPs are placed next to the step they inhibit.

704

705 **Fig. 2. Models of the effect of changes in the bacterial cell surface in CAMPs resistance.** CM,
706 cytoplasmic membrane; CW, cell wall; WTA, wall teichoic acids; LTA, lipoteichoic acids. CAMPs
707 are depicted as red stars.

708 A, cell envelope in the absence of D-alanylation of TAs and L-lysinylation of membrane
709 phospholipids. Local concentration of CAMPs is increased presumably by electrostatic
710 interactions with the cell envelope. CAMPs can reach the cell membrane and interact with
711 their targets.

712 B, electrostatic hindrance model for CAMP resistance. D-alanylation of TAs and L-lysinylation of
713 membrane phospholipids decrease the net negative charge of the cell envelope and the local
714 concentration of CAMPs.

715 C, electrostatic and steric hindrance model. D-alanylation of TAs modifies the cell wall
716 structure making it less permeable to CAMPs.

717 **Fig. 3. Schematic representation of the postulated models of action of ABC transporters**
718 **conferring CAMP resistance.**

719 A, LanFEG and BcrAB transporters. Transporters are shown in green, and ATP-hydrolysis and
720 substrate translocation are indicated by black solid and dashed arrows, respectively. CAMPs
721 are shown as red stars. 1) Transport assisted by NukH-type immunity proteins. 2) Binding of
722 CAMPs by NisI-type immunity proteins. 3) Hydrophobic vacuum-cleaner model of efflux
723 mechanism. 4) CAMPs (bacitracin) bind to the pyrophosphate group of UPP preventing its
724 dephosphorylation by undecaprenyl pyrophosphate phosphatase (UppP; pink pentagon); UPP
725 and UP molecules are shown schematically and dephosphorylation is indicated by a black
726 arrow. IM, immunity protein.

727 B, BceAB-type transporters. 5) Sensing, 6) resistance and 7) dual function transport systems.
728 Signaling between the transporters (green) and the TCS (blue) is indicated by a double-headed
729 black arrow. Phosphotransfer within TCS and gene activation are indicated by black arrows,
730 and the increased expression of transporter genes is indicated by straight dotted arrows. The
731 positions of promoters relative to genes were chosen arbitrarily. Likely dimerization of BceB-
732 type permease subunits is not shown for reasons of simplicity. HK, histidine kinase; RR,
733 response regulator.

734

735

Table 1: Summary of ABC transporters main characteristics. Based on (Gebhard, 2012).

	LanFEG	BcrAB^a	BceAB
Domain architecture	Permeases of 200–250 aa ^b and six TM ^b helices each	Permeases of approximately 230 aa with six predicted TM helices	Permease of approximately 650 aa and 10 TM helices, with a large – approx. 200 aa-extracellular domain located between helices VII and VIII
Direction of substrate transport	Export (the lantibiotic is removed from the cytoplasmic membrane to the culture supernatant) ^c	Unknown (export postulated) ^d	Unknown. Import suggested, followed by cytoplasmic enzymatic inactivation of the CAMP through degradation ^e
Associated proteins	Immunity proteins: LanI-type proteins (tethered to the membrane surface via an N-terminal lipoprotein anchor) and LanH-type proteins (contain three TM helices with the N-terminus located intracellularly)	Undecaprenyl-pyrophosphatase (UppP)	BceRS-like TCS ^b
Regulation^d	Mostly regulated by a TCS with prototypical periplasmic sensing HK ^{b,f} and OmpR family RR ^b . Others by XRE family transcriptional regulators	Mostly regulated by a TCS with IM-HK ^{b,f} and OmpR family RR. Others by XRE transcriptional regulators	BceRS-like TCS with IM-HK and OmpR family RR. Transporter regulating its own expression in response to CAMPs (see text for details)
Physiological role	Mostly involved in self-protection of lantibiotic producing strains (some are genetically associated with lantibiotic biosynthesis genes). Rarely, AMP resistance in non-producing strains.	Resistance against the cyclic AMP bacitracin in producing (self-protection) and non-producing strains	AMP resistance in non-producing strains
Substrates	Lantibiotics (nis, gall, epi, nuk, sub, etc.) ^g and dipeptide lantibiotics (lact) ^g	Cyclic AMP: bac ^g	Lantibiotics (nis, sub, gall, mer) ^g , cyclic AMPs (bac), lipodepsipeptides (end) ^g , glycopeptides (van, tei) ^g , peptides from the immune system of higher organisms like

^a For simplicity only the BcrAB transporters are included. The reader is referred to Gebhard 2012 (Gebhard, 2012) for additional information on YydJ.

^b aa: amino acids; TM: transmembrane; TCS: two component systems; HK: Histidine kinase; RR: Response regulator; IM-HK: intramembrane-sensing histidine kinase.

^c (Otto et al., 1998; Stein et al., 2003; Okuda et al., 2008)

^d (Gebhard, 2012)

^e (Rietkötter et al., 2008; Hiron et al., 2011)

^f (Mascher et al., 2006).

^g *act*: actagardine; *bac*: bacitracin; *bre*: brevinin; *end*: enduracidin; *epi*: epidermin; *gall*: gallidermin; *ind*: indolicidin; *lact*: lactacin 3147; *mer*: mersacidin; *nis*: nisin; *nuk*: nukacin; *ovi*: ovispirin; *ple*: plectasin; *sub*: subtilin; *tei*: teicoplanin; *van*: vancomycin.





