ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2010, p. 4658–4663 Vol. 54, No. 11
0066-4804/10/$12.00 doi:10.1128/AAC.00290-10

TelA Contributes to the Innate Resistance of *Listeria monocytogenes* to Nisin and Other Cell Wall-Acting Antibiotics▽

Barry Collins,[1] Susan Joyce,[1,2] Colin Hill,[1,2]* Paul D. Cotter,[2,3]* and R. Paul Ross[2,3]

Department of Microbiology, University College Cork, Cork, Ireland[1]; Alimentary Pharmabiotic Centre, Cork, Ireland[2]; and Teagasc, Moorepark Food Research Centre, Fermoy, Co. Cork, Ireland[3]

Received 26 February 2010/Returned for modification 25 June 2010/Accepted 12 August 2010

Nisin is a class I bacteriocin (lantibiotic), which is employed by the food and veterinary industries and exhibits potent activity against numerous pathogens. However, this activity could be further improved through the targeting and inhibition of factors that contribute to innate nisin resistance. Here we describe a novel locus, *lmo1967*, which is required for optimal nisin resistance in *Listeria monocytogenes*. The importance of this locus, which is a homologue of the tellurite resistance gene *telA*, was revealed after the screening of a *mariner* random mutant bank of *L. monocytogenes* for nisin-susceptible mutants. The involvement of *telA* in nisin resistance was confirmed through an analysis of a nonpolar deletion mutant. In addition to being 4-fold-more susceptible to nisin, the Δ*telA* strain was also 8-fold-more susceptible to gallidermin and 2-fold-more susceptible to cefuroxime, cefotaxime, bacitracin, and tellurite. This is the first occasion upon which *telA* has been investigated in a Gram-positive organism and also represents the first example of a link being established between a *telA* gene and resistance to cell envelope-acting antimicrobials.

Nisin is a ribosomally synthesized cationic antimicrobial peptide that has been employed commercially for over 50 years in the preservation of foodstuffs (22) and, more recently, as an antimastitis agent (15). It also exhibits great potency against a number of human clinical pathogens, including many multidrug-resistant strains (33), and as a consequence of the continually diminishing options available to clinicians when targeting such microorganisms, the application of nisin has been the subject of renewed attention. Nisin is the prototypical example of the class I group of bacteriocins, which are also known as lantibiotics by virtue of the presence of unusual posttranslationally introduced structures known as lanthionines (12). In addition to being the most thoroughly characterized lantibiotic, nisin is also an example of a cell envelope-acting antimicrobial, acting through a combination of inhibiting peptidoglycan synthesis and forming pores in the cell membrane of target cells (3, 4, 18, 45).

Despite the potency of nisin, there is evidence that suggests that its activity would be even greater were it not for factors that contribute to the innate resistance of some target microorganisms; the deletion of *virR* and *mprF* is known to result in 32- and 16-fold reductions in resistance to nisin in *Listeria monocytogenes* (9). For clarity, we discriminate between the mechanisms underpinning acquired resistance (resistance occurring in a formerly susceptible strain) and innate resistance (resistance intrinsically associated with particular genera or species). One example of a system contributing to innate resistance is DltA, which is required for the D-alanyl decoration of teichoic acid in the cell wall of many Gram-positive microorganisms. Its role in antimicrobial resistance was first noted when a disruption of *dltA* resulted in the sensitization of *Staphylococcus aureus* to the lantibiotic gallidermin as a consequence of a reduced capacity to repulse positively charged compounds (30). This susceptibility and, indeed, a susceptibility to a wider range of cationic antimicrobial peptides (CAMPs), including nisin, defensins, vancomycin, polymyxin B, and colistin, are also apparent in *dltA* mutants of *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Enterococcus faecalis*, and *Listeria monocytogenes* (14, 20, 25, 30, 35). An altered cell envelope charge, in this case due to the nonlysinylation of membrane phospholipids, is also the basis for the enhanced susceptibility of *mprF* mutants of *S. aureus*, *L. monocytogenes*, and *Bacillus anthracis* to nisin and other CAMPs (29, 36, 42). Unsurprisingly, eliminating the VirR regulator component of the two-component signal transduction system (VirRS) that regulates the expression of both *dltA* and *mprF* in *L. monocytogenes* also impacts susceptibility to CAMPs (23, 42). Other loci that play a role in the innate resistance of Gram-positive bacteria to nisin include *lisRK*, lmo0327, *pbp2229*, *sigB*, *graS*, *nsr*, and *abcAB* (1, 11, 16, 24, 34, 37, 40). There have also been a number of loci linked with acquired resistance to nisin through gene expression-based studies. An analysis of nisin-resistant mutants of *Lactococcus lactis* Il1403 revealed the increased expression of a number of different genes, including cell wall-related loci, operons involved in metabolism, as well as a number of genes involved in transport and stress responses (21). The contribution of some of these genes, i.e., *dltD*, *galKMT*, and *ahrC*, to nisin resistance was subsequently confirmed by using genetic knockouts. The involvement of another set of genes, *ysaBC*, was confirmed when overexpression was found to lead to increased nisin resistance (21). Expression studies have also established that the histidine kinase-encoding gene (lmo1021), a penicillin binding protein determinant (lmo2229), and a gene encoding a protein of unknown function

* Corresponding author. Mailing address for Colin Hill: Department of Microbiology, University College Cork, Cork, Ireland. Phone: 353-21-4901373. Fax: 353-21-4903101. E-mail: c.hill@ucc.ie. Mailing address for Paul D. Cotter: Teagasc, Moorepark Food Research Centre, Fermoy, Cork, Ireland. Phone: 353-25-42694. Fax: 353-25-42340. E-mail: paul.cotter@teagasc.ie.

▽ Published ahead of print on 16 August 2010.

TABLE 1. Strains, plasmids, and PCR primers used

Strain, primer, or plasmid	Relevant feature(s)[f]	Source or reference
Strains		
DH10B	Cloning host strain	Invitrogen
L. monocytogenes EGDe	Serotype 1/2a strain; genome sequenced	15a
Primers		
Lmo1967_PstI_SOEA	CT**CTGCAG**TGGAGCTAACTG[a]	This study
Lmo1967_SOEB	CACTTGGCTTGTTCTCGGTC	This study
Lmo1967_SOEC	GACCGAGAACAAGCCAAGTGACAAAACCCCACTCTTACG[b]	This study
Lmo1967_XbaI_SOED	CC**TCTAGA**ATGCTTGCGAC[c]	This study
Lmo1967_OutFor	CCAAGAGCGTAACAAAACGC	This study
Lmo1967_OutRev	GGAATGATTCATGCCTGTG	This study
Lmo1967_comp_NCO_F	CAAA**CCATGG**CCGAGAACAAGCC[d]	This study
Lmo1967_comp_PST_R	GGG**CTGCAG**TTATTTCATTTC[e]	This study
Marq207	GGCCACGCGTCGACTAGTACNNNNNNNNNNGTAAT	6
Marq208	GGCCACGCGTCGACTAGTAC	6
marq255	CAGTACAATCTGCTCTGATGCCGCATAGTT	6
marq256	TAGTTAAGCCAGCCCCGACACCCGCCAACA	6
marq257	CTTACAGACAAGCTGTGACCGTCT	6
PL95	ACATAATCAGTCCAAAGTAGATGC	21a
PL102	TATCAGACCTAACCCAAACCTTCC	21a
Plasmids		
pKSV7	Temperature-sensitive integration vector; Cm^r	38a
pIMK2	Integrative vector, overexpression, 6.2 kb; Kan^r	26
pMC38	*mariner* delivery vector	6

[a] The sequence in boldface type is the PstI restriction enzyme site.
[b] The underlined sequence is an overlap designed to complement primer Lmo1967_SOEB.
[c] The sequence in boldface type is the XbaI restriction enzyme site.
[d] The sequence in boldface type is the NcoI restriction enzyme site.
[e] The sequence in boldface type is the PstI restriction enzyme site.
[f] Primers are 5′ to 3′.

(lmo2487) are all upregulated in spontaneously nisin-resistant *L. monocytogenes* strains (16).

Here the screening of a *mariner* transposon bank of *L. monocytogenes* EGDe has resulted in the identification of a mutant that is susceptible to nisin as a consequence of the disruption of lmo1967, a gene homologous to tellurite resistance loci, designated *telA*, found in many members of the *Firmicutes* and *Proteobacteria*. This is the first occasion in which a *telA* gene has been associated with resistance to a cell envelope-acting antimicrobial and the first time that it has been studied in a Gram-positive bacterium. The study of this transposon mutant, and of another mutant in which the gene was removed in a nonpolar manner, revealed that *telA* also contributes to the pathogen's natural resistance to tellurite and to many cell envelope-acting antimicrobials, including gallidermin, bacitracin, cefuroxime, and cefotaxime.

MATERIALS AND METHODS

Strains, plasmids, and media. The bacterial strains, plasmids, and culture conditions used in this study are listed in Table 1. Strains were grown at 37°C with shaking unless otherwise stated. *L. monocytogenes* was grown aerobically in tryptic soy broth with yeast extract (TSB-YE). *Escherichia coli* cells were cultured in Luria-Bertani medium. Antibiotics were used at the following concentrations: erythromycin at 5 μg/ml, kanamycin at 50 μg/ml, and chloramphenicol (Cm) at 10 μg/ml.

Generation of a random mutant bank. A *mariner* random mutant bank of *L. monocytogenes* EGDe mutants was generated as described previously by Cao et al. (6). The pMC38 vector was used to generate the bank, in preference to pMC39, due to the high transformation efficiency of this vector in electrocompetent *L. monocytogenes* EGDe. Plasmid retention was estimated by plating the bank separately on TSB-YE agar plates containing either 5 μg/ml erythromycin or 10 μg/ml kanamycin. Plasmid retention was calculated by dividing the number of clones that retained the plasmid by the total number of clones. The Genetix (Hampshire, United Kingdom) Qpix2 XT robotic system was utilized to pick 7,000 *mariner* transposon-containing *L. monocytogenes* colonies and stock them in TBS-YE freezing buffer [TSB-YE supplemented with K_2HPO_4 (36 mM), KH_2PO_4 (13.2 mM), sodium citrate (1.7 mM), $MgSO_4$ (0.4 mM), $(NH_4)_2SO_4$ (6.8 mM), and 4.4% glycerol] in 384-well microtiter plates. To identify nisin-susceptible clones, the entire bank was replica plated onto tryptic soy agar with yeast extract (TSA-YE) containing 2 μg/ml Nisaplin (2.5% nisin; Danisco) using the Qpix robotic system. Plates were incubated for 24 h, and nisin-susceptible clones were identified on the basis of a lack of growth.

Identification of the site of transposon insertion. The site of transposon insertion was identified as described previously (6). Briefly, an "arbitrary" PCR was first performed by using primers Marq207 and Marq255. Reddymix Extensor PCR master mix (Therma Scientific, Waltham, MA) was utilized for PCRs according to the manufacturer's instructions. A 1/25 dilution of the product of this reaction was used as a template for a second PCR with primers Marq208 and Marq256. The resulting PCR products were sequenced by using primer Marq257 (MWG Eurofins, Ebersburg, Germany), and the location of the transposon insertion was revealed with the help of BLAST (Basic Local Alignment Search Tool) analysis (www.ncbi.nlm.nih.gov/BLAST/).

Construction of a nonpolar deletion mutant of *telA*. A nonpolar deletion of *telA* was generated by using the temperature-sensitive shuttle vector pKSV7 coupled with splicing by overlap extension (SOE) as described previously (10). Briefly, two regions up- and downstream of the *telA* gene were amplified by using telASoeAB and telASoeCD, respectively. These products were cleaned, mixed in equal proportions, and spliced through a second PCR. The resultant telAsoeAD product was digested with PstI and XbaI, cloned into pKSV7, and transformed into *E. coli* DH10β cells. Following sequencing of the telAsoeAD insert, this plasmid was introduced into electrocompetent *L. monocytogenes* EGDe (26), and chloramphenicol-resistant transformants were selected. Following serial subculturing at 42°C in TSB-YE with 10 μg/ml chloramphenicol, cells into which pKSV7-telAsoeAD had integrated, via homologous recombination, into the EGDe genome were selected for by plating onto TSA-YE at 42°C. Plasmid excision, via a second recombination event, and curing occurred spontane-

ously after continuous subculturing in TSB-YE at 30°C. Cultures were plated onto TSA-YE at 30°C, and chloramphenicol-sensitive colonies (i.e., excisants) were identified by replica plating onto TSA-YE and TSA-YE-Cm. Successful mutagenesis was confirmed by PCR using primers Lmo1967_OutFor and Lmo1967_OutRev.

Complementation of the Δ*telA* mutation. The complementation of the Δ*telA* deletion was achieved by amplifying (with primers lmo1967_comp_Nco_f and lmo1967_comp_pst_r) and cloning *telA* into the pIMK2 vector to generate pIMK2Lmo1967 and integrating this vector into the EGDe Δ*telA* mutant genome by using a procedure described previously (26). The integration of the vector in kanamycin-resistant colonies was confirmed by PCR using primers PL95 and PL102.

Nisin challenge assays. The growths of *L. monocytogenes* strains (2% inoculum in TSB-YE) in the presence of different concentrations of nisin (0, 100, 300, and 500 μg/ml of Nisaplin [2.5% nisin]; Danisco) were compared by monitoring the optical density at 600 nm (OD_{600}) with a Spectra Max 340 spectrophotometer (Molecular Devices, CA).

Antibiotic disk assays. The susceptibilities of *L. monocytogenes* strains to a variety of antibiotics were initially determined by antibiotic disk diffusion assays as described previously (11, 19). Briefly, stationary-phase cultures (16 h) were diluted to 10^7 CFU/ml and swabbed onto TSA-YE. Six-millimeter antibiotic disks (Oxoid) infused with specific antibiotics were placed onto the agar plates, and following overnight growth (16 h), the zones of inhibition were measured.

MICs. MICs in TSB-YE medium were determined as described previously (13). One-hundred-microliter volumes of 2-fold serial dilutions of the antimicrobial to be tested were added to the wells of a 96-well plate containing 100 μl of the target bacteria at a concentration of 10^5 CFU/ml. After 20 h (high-performance liquid chromatography [HPLC]-purified nisin A) or 16 h (other antimicrobials) of incubation, the MIC was read as the lowest concentration that resulted in an absence of visible growth.

RESULTS AND DISCUSSION

Generation and screening of a random *L. monocytogenes* mutant bank. A *mariner* random mutant bank of *L. monocytogenes* EGDe was generated as described previously by Cao et al. (6). Plasmid retention within the bank was estimated to be 0.2%, thereby establishing that transposon insertion was successful. Twenty clones were picked at random, and the number of insertion events in each was determined by Southern hybridization. In each case, only single insertions were seen. The bank was arrayed by using a Qpix II colony-picking robotic system, thus facilitating the rapid screening of 7,000 random mutants for clones that were susceptible to 2 mg/ml Nisaplin (2.5% nisin) in TSA-YE. Clones that did not grow on this medium after 24 h were selected for further examination. Clone C5 was identified as a consistently nisin-susceptible mutant when growth was analyzed in the presence of Nisaplin (2.5% nisin) (Fig. 1). The location of the transposon insertion site was identified at position 595 of the 1,200-bp lmo1967 by the *mariner* arbitrary PCR method (6).

***In silico* analysis of lmo1967.** Initial *in silico* analysis was carried out in the form of a PSI-blast search on the 399-amino-acid (aa) predicted product of lmo1967. Hits above identities of 23% and E values of 1e−17 were considered relevant. A total of 323 bacteria were found to possess homologues, primarily those from the phyla *Firmicutes* and *Proteobacteria*. Unsurprisingly, those present in other members of the *Firmicutes* most closely resembled the EGDe equivalent. lmo1967 is thought to form a two-gene operon with lmo1966, which encodes a putative member of the halogen hydrol superfamily (XpaC) (Fig. 2). Indeed, the loci corresponding to lmo1966 and lmo1967 are also found in *Listeria innocua*, *Listeria welshimeri*, and *Listeria grayi*, while the same genetic organization is also conserved across many other members of the *Firmicutes*

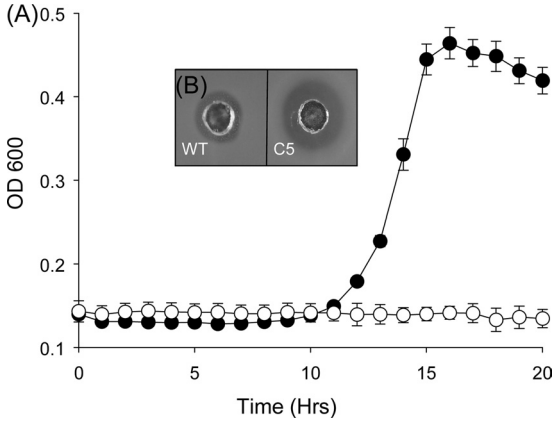

FIG. 1. (A) Kinetic growth assay showing impaired growth of EGDe transposon mutant C5 (open symbols) compared to the wild type (WT) (closed symbols) in the presence of 500 μg/ml Nisaplin (2.5% nisin). (B) Deferred antagonism assay showing increased susceptibility of C5 to Nisaplin.

and *Proteobacteria*. For *L. monocytogenes*, transcriptomics-based studies previously revealed that the expression of this operon is upregulated in stationary phase (and is SigB dependent) and in an intestinal environment but is downregulated in blood and brain heart infusion (BHI) broth (5, 44), while proteomics has established that the Lmo1967 protein is a member of the *L. monocytogenes* cell wall subproteome (38). Loci corresponding to *lmo1967* in other Gram-positive microorganisms are known by a variety of other names, including *klaB*, *ynhC*, *yaaN*, and *yceH*. In *Bacillus subtilis*, *yaaN* is induced under stress from the cationic antimicrobial compound poly-L-lysine (32) by both salt and alkaline shock and is part of the SigW regulon (31, 46). Notably, *ynhC* is also upregulated in *L. lactis* in response to nisin stress (21). The closest homologue of lmo1967 for which a function has been assigned is the *Rhodobacter sphaeroides* gene *telA* (38% homology), and thus, lmo1967 was redesignated *telA* (28). *telA* has been characterized on the basis of being required for full resistance to the toxic compound tellurite. Tellurite compounds were trialed as antimicrobials in the first half of the 20th century, showing efficacy against tuberculosis, leprosy, and syphilis. Presently, the main application for tellurite compounds is as an ingredient in selective media, where tellurite resistance is characterized by black color development in resistant colonies (41). A number of genes are known to be involved in tellurite resistance, including *cysM*, *katA*, *sodAB*, and *soxS* as well as a number of operons that, to date, have been associated only with tellurite resistance (*teh*, *ter*, *tel*, *trg*, and *tmp*) (8).

Creation and characterization of the *L. monocytogenes* EGDe Δ*telA* mutant strain. To ensure that the nisin-sensitive phenotype of mutant C5 was a consequence of the disruption of *telA*, a nonpolar deletion mutant of *telA* was created and studied. This ruled out the possibility of the nisin-sensitive phenotype being due to polar effects in the transposon mutant. The Δ*telA* mutant was created by deleting 1,150 bp of the 1,200-bp gene using the SOE-PCR procedure, the temperature-sensitive shuttle vector pKSV7, and double-crossover homologous recombination. The susceptibility of the Δ*telA* mutant was initially assessed by growing the strain in broth containing 100 to

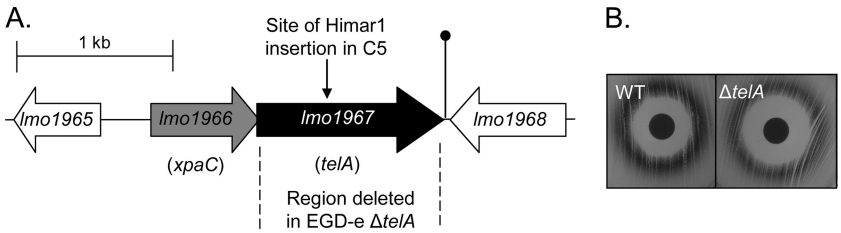

FIG. 2. (A) Genetic organization of the point of insertion of the himar1 transposon. The dashed line shows the proportion of the gene deleted. The lollipop symbol indicates a putative terminator. All genes in the schematic are drawn to scale. (B) Disk diffusion assay showing a potassium tellurite-impregnated disk on plates spread with wild-type *L. monocytogenes* and the *L. monocytogenes* Δ*telA* mutant. An increased zone of inhibition indicates increased susceptibility in the Δ*telA* mutant. Lighter shades of the zone of tellurite reduction indicate an impaired ability to reduce tellurite to elemental tellurium.

500 μg/ml Nisaplin (2.5% nisin) (Fig. 3). It was apparent that the growth of the Δ*telA* mutant was dramatically affected by the presence of Nisaplin, being most apparent in the presence of 500 μg/ml of the preservative when the mutant failed to grow. This contrasted with the vigorous growth of the parental strain EGDe under these conditions (Fig. 3). To ensure that this susceptibility was as a consequence of the nisin A present in Nisaplin rather than, for example, its NaCl content, MIC determination assays with pure nisin A were performed. From these experiments, it was apparent that the Δ*telA* mutant was 4-fold-more susceptible (MIC = 6 μg/ml) than the parent (MIC = 25 μg/ml) after 20 h of growth (see Table 3). The Δ*telA* mutation was complemented by cloning *telA* behind the strong promoter of plasmid pIMK2 and integrating the vector into the mutant strain (26) to generate the EGDe Δ*telA*::*telA* strain. This complemented strain no longer exhibited a nisin-susceptible phenotype (Fig. 3).

Antimicrobial susceptibility assays. Given the description of *telA* of other microorganisms as a tellurite resistance locus, the susceptibility of the Δ*telA* mutant to potassium tellurite was also assessed. The investigations established that the Δ*telA* strain is 2-fold-more susceptible to potassium tellurite (see Table 3). Notably, growth on tellurite-containing agar revealed that the mutant appeared to still be able to process tellurite to some extent, as evidenced by a characteristic black precipitate. However, the observation that the associated precipitate was less obvious than that produced by the parent strain suggests an impaired ability to reduce tellurite to elemental tellurium (Fig. 2B). *L. monocytogenes* EGDe was subjected to an *in silico* analysis to screen for the presence of homologues of the other four known tellurite resistance genetic determinants (41). However, none were identified, suggesting that the genes responsible for residual tellurite resistance mechanisms are novel. The frequency with which tellurite resistance mecha-

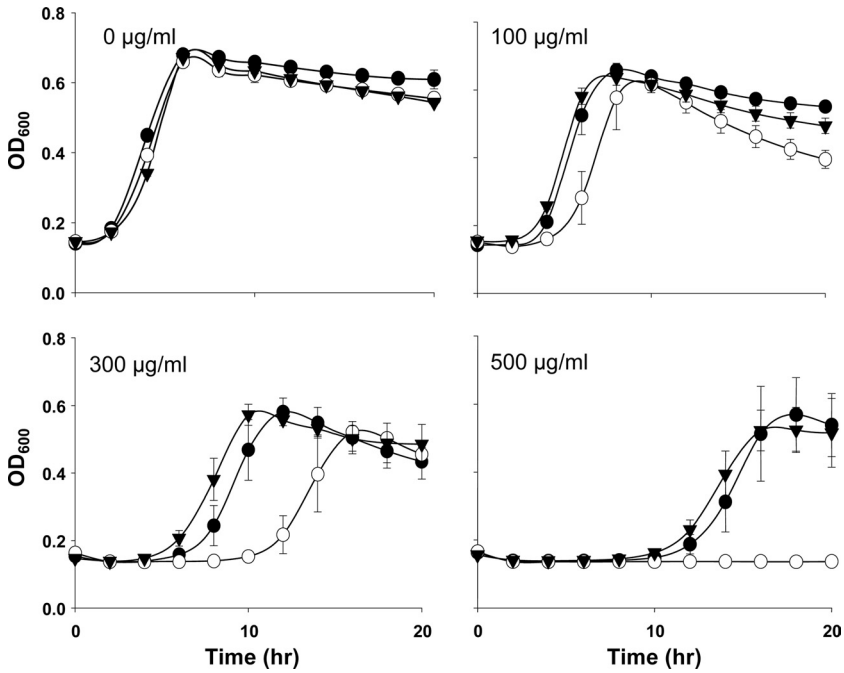

FIG. 3. Growth of EGDe and the Δ*telA* and Δ*telA*::*telA* mutants in the presence of antimicrobial agents. Shown is the growth of EGDe (closed circles), the EGDe Δ*telA* mutant (open circles), and the EGDe Δ*telA*::*telA* mutant (closed triangles) in TSB-YE with 100 to 500 μg/ml of Nisaplin (2.5% nisin). Error bars are standard deviations from the means of data from triplicate experiments.

TABLE 2. Antibiotic disk assays

Antibiotic	Mean size of zone of inhibition (mm) ± SD	
	WT	TelA mutant
Cefuroxime	21.1 ± 0.2	24.4 ± 0.3
Bacitracin	12.5 ± 0.3	15.1 ± 0.4
Oxacillin	10.7 ± 0.3	12.3 ± 0.2
Cefotaxime	21.3 ± 0.3	23.7 ± 0.3
Methicillin	25.6 ± 0.2	26.7 ± 0.4

TABLE 3. MICs[a]

Antimicrobial	MIC (μg/ml)	
	WT	TelA mutant
Nisin	25.14	6.29
Gallidermin	1.97	0.246
Bacitracin	256	128
Cefotaxime	2	1
Tellurite	38	19
Cefuroxime	2.87	1.44

[a] Results represent the averages of data from three independent experiments. As the values were identical in each case, the standard deviation is zero. MIC data are derived from a 2-fold dilution series. For nisin and gallidermin the μg/ml value is extrapolated from a μM value; e.g., a nisin concentration of 7.5 μM is equivalent to 25.14 μg/ml.

nisms are located across different bacterial species is a curiosity, especially as tellurite itself is a very rare element in the environment (the 75th most abundant metal). Thus, it is anticipated that it will ultimately be established that tellurite-specific stress responses are very rare (should they exist at all) and that resistance is most frequently mediated through more-general stress response mechanisms. On this basis, the ability of the Δ*telA* mutant to withstand exposure to a number of other antimicrobials, relative to the parental strain, was also assessed. In order to include as many antimicrobials as possible, this screen initially took place in the form of antimicrobial disk agar-based assays. However, in instances where differences in relative susceptibility were observed, subsequent MIC determination studies were performed. The antibiotic disks employed included cefuroxime, cefotaxime, rifampin, vancomycin, gentamicin, novobiocin, tetracycline (30 μg), trimethoprim-sulfamethoxazole (25 μg), bacitracin, erythromycin, ampicillin (10 μg), methicillin, penicillin G (5 μg), and oxacillin (1 μg). These assays revealed that the Δ*telA* mutant exhibits enhanced susceptibility to oxacillin, cefuroxime, cefotaxime, methicillin, and bacitracin (Table 2). Further MIC-based analyses revealed that this corresponded to a 2-fold-greater susceptibility to the beta-lactam antibiotics cefuroxime and cefotaxime and to the non-beta-lactam antibiotic bacitracin and a dramatic 8-fold-increased susceptibility to the lantibiotic gallidermin (Table 3). MIC analysis did not reveal differences in susceptibility for oxacillin and methicillin. It is thus apparent that TelA contributes greatly to the innate resistance of *L. monocytogenes* EGDe to a number of important cell envelope-acting antimicrobials.

Despite its broad distribution, this is the first occasion upon which a *telA* homologue in a Gram-positive bacterium has been investigated in depth. Even more importantly, while an analysis of transcriptomics data reveals that the level of expression of *telA* homologues is increased in a variety of bacteria in response to exposure to nisin, poly-L-lysine, vancomycin, and daptomycin (7, 21, 27, 32), this is the first time that antimicrobial susceptibility has been noted for a Δ*telA* mutant.

Here TelA has been identified as a novel mediator of resistance to a number of cell envelope-acting antimicrobials, including the lantibiotics nisin and gallidermin, the beta-lactams cefuroxime and cefotaxime, as well as bacitracin. Both lantibiotics are inhibitors of peptidoglycan synthesis as a consequence of binding lipid II, and in addition, nisin and, on occasion, gallidermin can also form pores in the cell membrane of target microorganisms (2). The beta-lactam antibiotics inhibit the cross-linking of peptidoglycan chains, while bacitracin affects the dephosphorylation of bactophenol during peptidoglycan synthesis (39, 43). While bacitracin is not usually employed as an anti-*Listeria* agent, the identification of a locus that contributes to the innate resistance of *L. monocytogenes* to nisin and cephalosporins is notable in light of their respective use as food preservatives and clinical antibiotics. Nisin is the only natural antibacterial preservative that has been approved for food applications, and thus, its frequently limited activity against *L. monocytogenes* is problematic; however, its efficacy increases when integrated as part of a hurdle approach to food protection. Cephalosporins are often used as the antibiotics of first choice when treating infections of unknown etiology, and thus, the high innate resistance of *L. monocytogenes* to this class of antibiotics may also have serious consequences. Curiously, this is not the first occasion upon which nisin and cephalosporin resistance have been linked, as the *L. monocytogenes* Δ*lisK* mutant exhibits enhanced nisin resistance but greater susceptibility to cephalosporins (11), while mutants that are spontaneously resistant to the lantibiotic lacticin 3147 have also shown increased cephalosporin susceptibility (17). A further understanding of this phenomenon could lead to the development of strategies to disrupt these mechanisms with a view to increasing the susceptibility of the pathogen to these antimicrobials.

ACKNOWLEDGMENTS

We acknowledge Nicola Curtis for her assistance in the screening of the *L. monocytogenes* mutant bank. We thank Hélène Marquis for providing plasmid pMC38, Joss Delves-Broughton (Danisco) for Nisaplin powder, and Friedrich Götz for purified gallidermin.

This work was supported by the Irish Department of Agriculture by a Food Institutional Research Measure (FIRM) grant (Funlac).

REFERENCES

1. **Begley, M., C. Hill, and R. P. Ross.** 2006. Tolerance of *Listeria monocytogenes* to cell envelope-acting antimicrobial agents is dependent on SigB. Appl. Environ. Microbiol. **72:**2231–2234.
2. **Bonelli, R. R., T. Schneider, H. G. Sahl, and I. Wiedemann.** 2006. Insights into in vivo activities of lantibiotics from gallidermin and epidermin mode-of-action studies. Antimicrob. Agents Chemother. **50:**1449–1457.
3. **Breukink, E., I. Wiedemann, C. van Kraaij, O. P. Kuipers, H. Sahl, and B. de Kruijff.** 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. Science **286:**2361–2364.
4. **Brotz, H., M. Josten, I. Wiedemann, U. Schneider, F. Gotz, G. Bierbaum, and H. G. Sahl.** 1998. Role of lipid-bound peptidoglycan precursors in the formation of pores by nisin, epidermin and other lantibiotics. Mol. Microbiol. **30:**317–327.
5. **Camejo, A., C. Buchrieser, E. Couve, F. Carvalho, O. Reis, P. Ferreira, S. Sousa, P. Cossart, and D. Cabanes.** 2009. In vivo transcriptional profiling of *Listeria monocytogenes* and mutagenesis identify new virulence factors involved in infection. PLoS Pathog. **5:**e1000449.
6. **Cao, M., A. P. Bitar, and H. Marquis.** 2007. A *mariner*-based transposition system for *Listeria monocytogenes*. Appl. Environ. Microbiol. **73:**2758–2761.

7. **Cao, M., T. Wang, R. Ye, and J. D. Helmann.** 2002. Antibiotics that inhibit cell wall biosynthesis induce expression of the Bacillus subtilis sigma(W) and sigma(M) regulons. Mol. Microbiol. **45:**1267–1276.
8. **Chasteen, T. G., D. E. Fuentes, J. C. Tantalean, and C. C. Vasquez.** 2009. Tellurite: history, oxidative stress, and molecular mechanisms of resistance. FEMS Microbiol. Rev. **33:**820–832.
9. **Collins, B., N. Curtis, P. D. Cotter, C. Hill, and R. P. Ross.** 2010. The ABC transporter AnrAB contributes to the innate resistance of *Listeria monocytogene*s to nisin, bacitracin, and various β-lactam antibiotics. Antimicrob. Agents Chemother. **54:**4416–4423.
10. **Cotter, P. D., N. Emerson, C. G. Gahan, and C. Hill.** 1999. Identification and disruption of *lisRK*, a genetic locus encoding a two-component signal transduction system involved in stress tolerance and virulence in Listeria monocytogenes. J. Bacteriol. **181:**6840–6843.
11. **Cotter, P. D., C. M. Guinane, and C. Hill.** 2002. The LisRK signal transduction system determines the sensitivity of *Listeria monocytogenes* to nisin and cephalosporins. Antimicrob. Agents Chemother. **46:**2784–2790.
12. **Cotter, P. D., C. Hill, and R. P. Ross.** 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. **3:**777–788.
13. **Draper, L. A., K. Grainger, L. H. Deegan, P. D. Cotter, C. Hill, and R. P. Ross.** 2009. Cross-immunity and immune mimicry as mechanisms of resistance to the lantibiotic lacticin 3147. Mol. Microbiol. **71:**1043–1054.
14. **Fabretti, F., C. Theilacker, L. Baldassarri, Z. Kaczynski, A. Kropec, O. Holst, and J. Huebner.** 2006. Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides. Infect. Immun. **74:**4164–4171.
15. **Fernandez, L., S. Delgado, H. Herrero, A. Maldonado, and J. M. Rodriguez.** 2008. The bacteriocin nisin, an effective agent for the treatment of staphylococcal mastitis during lactation. J. Hum. Lact. **24:**311–316.

15a. **Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Duchaud, L. Durant, O. Dussurget, K. D. Entian, H. Fsihi, F. Garcia-del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L. M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueno, A. Maitournam, J. M. Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J. C. Perez-Diaz, R. Purcell, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J. A. Vazquez-Boland, H. Voss, J. Wehland, and P. Cossart.** 2001. Comparative genomics of *Listeria* species. Science **294:**849–852.

16. **Gravesen, A., K. Sorensen, F. M. Aarestrup, and S. Knochel.** 2001. Spontaneous nisin-resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. Microb. Drug Resist. **7:**127–135.
17. **Guinane, C. M., P. D. Cotter, C. Hill, and R. P. Ross.** 2006. Spontaneous resistance in *Lactococcus lactis* IL1403 to the lantibiotic lacticin 3147. FEMS Microbiol. Lett. **260:**77–83.
18. **Hasper, H. E., N. E. Kramer, J. L. Smith, J. D. Hillman, C. Zachariah, O. P. Kuipers, B. de Kruijff, and E. Breukink.** 2006. An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. Science **313:**1636–1637.
19. **Kallipolitis, B. H., H. Ingmer, C. G. Gahan, C. Hill, and L. Sogaard-Andersen.** 2003. CesRK, a two-component signal transduction system in *Listeria monocytogenes*, responds to the presence of cell wall-acting antibiotics and affects beta-lactam resistance. Antimicrob. Agents Chemother. **47:**3421–3429.
20. **Kovacs, M., A. Halfmann, I. Fedtke, M. Heintz, A. Peschel, W. Vollmer, R. Hakenbeck, and R. Bruckner.** 2006. A functional *dlt* operon, encoding proteins required for incorporation of D-alanine in teichoic acids in Gram-positive bacteria, confers resistance to cationic antimicrobial peptides in *Streptococcus pneumoniae*. J. Bacteriol. **188:**5797–5805.
21. **Kramer, N. E., S. A. van Hijum, J. Knol, J. Kok, and O. P. Kuipers.** 2006. Transcriptome analysis reveals mechanisms by which *Lactococcus lactis* acquires nisin resistance. Antimicrob. Agents Chemother. **50:**1753–1761.

21a. **Lauer, P., M. Y. Chow, M. J. Loessner, D. A. Portnoy, and R. Calendar.** 2002. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. J. Bacteriol. **184:**4177–4186.

22. **Lubelski, J., R. Rink, R. Khusainov, G. N. Moll, and O. P. Kuipers.** 2008. Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. Cell Mol. Life Sci. **65:**455–476.
23. **Mandin, P., H. Fsihi, O. Dussurget, M. Vergassola, E. Milohanic, A. Toledo-Arana, I. Lasa, J. Johansson, and P. Cossart.** 2005. VirR, a response regulator critical for *Listeria monocytogenes* virulence. Mol. Microbiol. **57:**1367–1380.
24. **Margolles, A., A. B. Florez, J. A. Moreno, D. van Sinderen, and C. G. de los Reyes-Gavilan.** 2006. Two membrane proteins from *Bifidobacterium breve* UCC2003 constitute an ABC-type multidrug transporter. Microbiology **152:**3497–3505.
25. **May, J. J., R. Finking, F. Wiegeshoff, T. T. Weber, N. Bandur, U. Koert, and M. A. Marahiel.** 2005. Inhibition of the D-alanine:D-alanyl carrier protein ligase from *Bacillus subtilis* increases the bacterium's susceptibility to antibiotics that target the cell wall. FEBS J. **272:**2993–3003.
26. **Monk, I. R., C. G. Gahan, and C. Hill.** 2008. Tools for functional post-genomic analysis of *Listeria monocytogenes*. Appl. Environ. Microbiol. **74:**3921–3934.
27. **Muthaiyan, A., J. A. Silverman, R. K. Jayaswal, and B. J. Wilkinson.** 2008. Transcriptional profiling reveals that daptomycin induces the *Staphylococcus aureus* cell wall stress stimulon and genes responsive to membrane depolarization. Antimicrob. Agents Chemother. **52:**980–990.
28. **O'Gara, J. P., M. Gomelsky, and S. Kaplan.** 1997. Identification and molecular genetic analysis of multiple loci contributing to high-level tellurite resistance in *Rhodobacter sphaeroides* 2.4.1. Appl. Environ. Microbiol. **63:**4713–4720.
29. **Peschel, A., R. W. Jack, M. Otto, L. V. Collins, P. Staubitz, G. Nicholson, H. Kalbacher, W. F. Nieuwenhuizen, G. Jung, A. Tarkowski, K. P. van Kessel, and J. A. van Strijp.** 2001. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. J. Exp. Med. **193:**1067–1076.
30. **Peschel, A., M. Otto, R. W. Jack, H. Kalbacher, G. Jung, and F. Gotz.** 1999. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J. Biol. Chem. **274:**8405–8410.
31. **Petersohn, A., M. Brigulla, S. Haas, J. D. Hoheisel, U. Volker, and M. Hecker.** 2001. Global analysis of the general stress response of *Bacillus subtilis*. J. Bacteriol. **183:**5617–5631.
32. **Pietiainen, M., M. Gardemeister, M. Mecklin, S. Leskela, M. Sarvas, and V. P. Kontinen.** 2005. Cationic antimicrobial peptides elicit a complex stress response in *Bacillus subtilis* that involves ECF-type sigma factors and two-component signal transduction systems. Microbiology **151:**1577–1592.
33. **Piper, C., L. A. Draper, P. D. Cotter, R. P. Ross, and C. Hill.** 2009. A comparison of the activities of lacticin 3147 and nisin against drug-resistant *Staphylococcus aureus* and *Enterococcus* species. J. Antimicrob. Chemother. **64:**546–551.
34. **Popowska, M., and Z. Markiewicz.** 2006. Characterization of *Listeria monocytogenes* protein Lmo0327 with murein hydrolase activity. Arch. Microbiol. **186:**69–86.
35. **Poyart, C., E. Pellegrini, M. Marceau, M. Baptista, F. Jaubert, M. C. Lamy, and P. Trieu-Cuot.** 2003. Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic cells. Mol. Microbiol. **49:**1615–1625.
36. **Samant, S., F. F. Hsu, A. A. Neyfakh, and H. Lee.** 2009. The *Bacillus anthracis* protein MprF is required for synthesis of lysylphosphatidylglycerols and for resistance to cationic antimicrobial peptides. J. Bacteriol. **191:**1311–1319.
37. **Sass, P., and G. Bierbaum.** 2009. Native *graS* mutation supports the susceptibility of *Staphylococcus aureus* strain SG511 to antimicrobial peptides. Int. J. Med. Microbiol. **299:**313–322.
38. **Schaumburg, J., O. Diekmann, P. Hagendorff, S. Bergmann, M. Rohde, S. Hammerschmidt, L. Jansch, J. Wehland, and U. Karst.** 2004. The cell wall subproteome of *Listeria monocytogenes*. Proteomics **4:**2991–3006.

38a. **Smith, K., and P. Youngman.** 1992. Use of a new integrational vector to investigate compartment-specific expression of the *Bacillus subtilis spoIIM* gene. Biochimie **74:**705–711.

39. **Stone, K. J., and J. L. Strominger.** 1971. Mechanism of action of bacitracin: complexation with metal ion and C 55-isoprenyl pyrophosphate. Proc. Natl. Acad. Sci. U. S. A. **68:**3223–3227.
40. **Sun, Z., J. Zhong, X. Liang, J. Liu, X. Chen, and L. Huan.** 2009. Novel mechanism for nisin resistance via proteolytic degradation of nisin by the nisin resistance protein NSR. Antimicrob. Agents Chemother. **53:**1964–1973.
41. **Taylor, D. E.** 1999. Bacterial tellurite resistance. Trends Microbiol. **7:**111–115.
42. **Thedieck, K., T. Hain, W. Mohamed, B. J. Tindall, M. Nimtz, T. Chakraborty, J. Wehland, and L. Jansch.** 2006. The MprF protein is required for lysinylation of phospholipids in listerial membranes and confers resistance to cationic antimicrobial peptides (CAMPs) on *Listeria monocytogenes*. Mol. Microbiol. **62:**1325–1339.
43. **Tipper, D. J.** 1985. Mode of action of beta-lactam antibiotics. Pharmacol. Ther. **27:**1–35.
44. **Toledo-Arana, A., O. Dussurget, G. Nikitas, N. Sesto, H. Guet-Revillet, D. Balestrino, E. Loh, J. Gripenland, T. Tiensuu, K. Vaitkevicius, M. Barthelemy, M. Vergassola, M. A. Nahori, G. Soubigou, B. Regnault, J. Y. Coppee, M. Lecuit, J. Johansson, and P. Cossart.** 2009. The *Listeria* transcriptional landscape from saprophytism to virulence. Nature **459:**950–956.
45. **Wiedemann, I., R. Benz, and H. G. Sahl.** 2004. Lipid II-mediated pore formation by the peptide antibiotic nisin: a black lipid membrane study. J. Bacteriol. **186:**3259–3261.
46. **Wiegert, T., G. Homuth, S. Versteeg, and W. Schumann.** 2001. Alkaline shock induces the *Bacillus subtilis* sigma(W) regulon. Mol. Microbiol. **41:**59–71.