Received: 28 July 2023 | Revised: 20 September 2023 | Accepted: 22 September 2023

DOI: 10.1111/mmi.15178

RESEARCH ARTICLE

WILEY

Tartrolon sensing and detoxification by the *Listeria monocytogenes timABR* resistance operon

Tim Engelgeh[1] | Jennifer Herrmann[2,3,4] | Rolf Jansen[5] | Rolf Müller[2,3,4] | Sven Halbedel[1,6]

[1]FG11 Division of Enteropathogenic Bacteria and Legionella, Robert Koch Institute, Wernigerode, Germany

[2]Department of Microbial Natural Products, Helmholtz Centre for Infection Research, Saarland University, Saarbrücken, Germany

[3]Department of Pharmaceutical Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarland University, Saarbrücken, Germany

[4]German Centre for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Braunschweig, Germany

[5]Department of Microbial Drugs, Helmholtz Centre for Infection Research, Braunschweig, Germany

[6]Institute for Medical Microbiology and Hospital Hygiene, Otto von Guericke University Magdeburg, Magdeburg, Germany

Correspondence
Sven Halbedel, FG11 Division of Enteropathogenic Bacteria and Legionella, Robert Koch Institute, Burgstrasse 37, D-38855 Wernigerode, Germany.
Email: halbedels@rki.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: HA6830/2-1

Abstract

Listeria monocytogenes is a foodborne bacterium that naturally occurs in the soil. Originating from there, it contaminates crops and infects farm animals and their consumption by humans may lead to listeriosis, a systemic life-threatening infectious disease. The adaptation of *L. monocytogenes* to such contrastive habitats is reflected by the presence of virulence genes for host infection and other genes for survival under environmental conditions. Among the latter are ABC transporters for excretion of antibiotics produced by environmental competitors; however, most of these transporters have not been characterized. Here, we generated a collection of promoter-*lacZ* fusions for genes encoding ABC-type drug transporters of *L. monocytogenes* and screened this reporter strain collection for induction using a library of natural compounds produced by various environmental microorganisms. We found that the *timABR* locus (*lmo1964-lmo1962*) was induced by the macrodiolide antibiotic tartrolon B, which is synthesized by the soil myxobacterium *Sorangium cellulosum*. Tartrolon B resistance of *L. monocytogenes* was dependent on *timAB*, encoding the ATPase and the permease component of a novel ABC transporter. Moreover, transplantation of *timAB* was sufficient to confer tartrolon B resistance to *Bacillus subtilis*. Expression of the *timABR* locus was found to be auto-repressed by the TimR repressor, whose repressing activity was lost in the presence of tartrolon B. We also demonstrate that tartrolon sensitivity was suppressed by high external potassium concentrations, suggesting that tartrolon acts as potassium ionophore. Our results help to map the ecological interactions of an important human pathogen with its co-residing species within their joint natural reservoir.

KEYWORDS
boromycin, boron, macrodiolide, MDR transporter, transcriptional repressor

1 | INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis, a dreaded foodborne infection in humans and farm animals. The bacterium belongs to the *Bacillota* phylum (formerly known as firmicutes) and can be found in different environmental habitats including the soil, surface waters, plant surfaces and the gut of various animals (Esteban et al., 2009; Freitag et al., 2009; Schoder et al., 2022; Truong et al., 2021). Contamination of crops and colonization of farm animals is the gateway for *L. monocytogenes* to be carried over into food production facilities, where the bacterium may colonize equipment, forms biofilms and finally contaminates food items (Quereda et al., 2021). After ingestion, *L. monocytogenes* is capable to breach the gut epithelium and to enter the bloodstream, but this early stage of the infection is normally brought under control by the immune system (Vazquez-Boland et al., 2001). However, the infection may spread to secondary organs in vulnerable risk groups, which then results in invasive listeriosis associated with high case fatality rates (Charlier et al., 2017; Wilking et al., 2021).

A combination of virulence factors and their regulatory elements orchestrates survival of *L. monocytogenes* in the infected host, while general and specific stress response genes are induced to cope with harmful conditions that prevail in environmental habitats (Quereda et al., 2021; Tiensuu et al., 2019). Among the latter are multidrug resistance (MDR) transporters excreting toxic compounds such as bile or ethidium bromide as well as antibiotics (Lubelski et al., 2007).

L. monocytogenes MDR transporters with known substrate specificities belong to at least four different classes: The major facilitator superfamily (MFS) transporters such as MdrL (for export of macrolides and ethidium bromide) and Lde (fluoroquinolones, ethidium bromide) use the proton motive force (PMF) as energy source to drive compound extrusion (Godreuil et al., 2003; Lubelski et al., 2007; Mata et al., 2000). Likewise, QacH, a representative of the small MDR (SMR) transporters, is PMF-dependent; it transports quaternary ammonium compounds out of the cell (Müller et al., 2014). Multidrug and toxic compound extrusion (MATE) transporters, such as *L. monocytogenes* FepA, also exploit transmembrane electrochemical (H^+ or Na^+) gradients but for export of fluoroquinolones (Guerin et al., 2014; Kuroda & Tsuchiya, 2009). The ATP binding cassette (ABC) type MDR transporters constitute a fourth but different class as they directly use ATP hydrolysis for compound extrusion. Transporters of this class consist of an ATPase coupled to a permease unit either separated on two polypeptides or fused into a single protein (Lubelski et al., 2007). Even though systems of this type are frequently regarded as MDR transporters, it often remains questionable whether they really aid in the transport of different compounds or whether they are rather SDR (single drug resistance) transporters (Wendlandt et al., 2015; Young & Holland, 1999).

Several *L. monocytogenes* ABC transporters are known to contribute to toxic compound excretion such as BilEAB (bile), AnrAB (bacitracin, nisin, cephalosporins) or LieAB (aurantimycin) (Collins et al., 2010; Hauf et al., 2019, 2021; Jiang et al., 2019; Sleator et al., 2005). Many more ABC-type transporters possibly related to compound export are encoded in the genome, however, most of them have not been characterized. We originally identified the *lieAB* genes while searching for target genes of the LftR transcriptional repressor (Hauf et al., 2019; Kaval et al., 2015). LftR is a PadR-type repressor that silences expression of the *lieAB* genes (Hauf et al., 2021). Repression of *lieAB* transcription is relieved upon aurantimycin exposure in a process that requires LftS, a co-regulator of LftR, and induction of LieAB production then confers aurantimycin resistance (Hauf et al., 2019, 2021). Whether aurantimycin is sensed by LftR directly or in complex with LftS is currently not clear (Hauf et al., 2021). Aurantimycin is a cyclic hexadepsipeptide, produced by soil-dwelling *Streptomyces aurantiacus* which forms pores in lipid membranes (Gräfe et al., 1995; Grigoriev et al., 1995). Bacitracin (the substrate of AnrAB) is a non-ribosomally synthesized macrocyclic peptide produced by several *Bacillus licheniformis* strains (Aase et al., 2000; Kopp & Marahiel, 2007), and nisin (another AnrAB substrate) is a gene-encoded and lanthionine ring-containing antimicrobial peptide produced by *Lactococcus lactis* (Lubelski et al., 2008). Like aurantimycin-producing *S. aurantiacus* that can be isolated from soil (Saraylou et al., 2021; Vijayabharathi et al., 2011), *B. licheniformis* (bacitracin producer) and *L. lactis* (nisin producer) are found in similar environmental niches (soil, plant surfaces) as *L. monocytogenes* (Cavanagh et al., 2015; Scheldeman et al., 2006). Moreover, expression of the *anrAB* ABC transporter genes is also induced by the same substances that are exported by their gene products (Jiang et al., 2019), as reported for the *lieAB* genes (Hauf et al., 2019). However, control of *anrAB* expression is mediated by the VirRS two-component system in conjunction with VirAB, a compound sensing ABC transporter (Jiang et al., 2019) rather than by a transcriptional repressor.

We here describe the discovery of a novel *L. monocytogenes* ABC transporter, named TimAB. We show that TimAB contributes to the resistance against tartrolon antibiotics, which represent another group of antimicrobial compounds produced by soil-dwelling microorganisms. Furthermore, we show how TimAB production is controlled by the tartrolon-responsive transcriptional regulator TimR. Our results illustrate the tight exposure of an important human pathogen to a diverse spectrum of microbial competitors in its natural reservoir.

2 | RESULTS

2.1 | Weakly transcribed MDR transporter genes in *L. monocytogenes* EGD-e

174 genes belonging to 104 putative or known ABC transporters are currently listed for *L. monocytogenes* EGD-e in the TransportDB database (Ren et al., 2004, 2007). Among them, there are 23 uncharacterized ABC transporters either directly classified as MDR transporters or as being potentially involved in excretion of antibiotics (Table 1). Previously published RNA-Seq data from our lab (Hauf et al., 2019) indicated that most of these transporter genes are not expressed under standard laboratory conditions (Table 1).

TABLE 1 ABC transporter genes in *L. monocyctogenes* EGD-e with possible functions in compound excretion (according to http://www.membranetransport.org).

ATPase	Permease	Operon[a]	TPM[b]
lmo0107[c]		*lmo0108 lmo0107*	2
lmo0108[c]			2
lmo0194	*lmo0195*	*lmo0193 lmo0194 lmo0195*	50
lmo0607[c]		*lmo0606 lmo0607 lmo0608*	51
lmo0608[c]			66
lmo0667	*lmo0668*	*lmo0667 lmo0668*[d]	60
lmo0742	*lmo0743*	*lmo0741 lmo0742 lmo0743 lmo0744 lmo0745*	5
lmo0744[c]			2
lmo0925[c]		*lmo0923 lmo0924 lmo0925 lmo0926*	30
lmo0986	*lmo0987*	*lmo0984 lmo0985 lmo0986 lmo0987*[d]	4
lmo1636	*lmo1637*	*lmo1635 lmo1636 lmo1637*	178
lmo1651[c]		*lmo1652 lmo1651 lmo1650 lmo1649 lmo1648*	28
lmo1652[c]			23
lmo1724	*lmo1723*	*lmo1725 lmo1724 lmo1723*	22
timA	*timB*	*lmo1964 (timA) lmo1963 (timB) lmo1962 (timR)*	15
lmo2215	*lmo2214*	*lmo2215 lmo2214*	97
lmo2227	*lmo2226*	*lmo2228 lmo2227 lmo2226*	14
lmo2240	*lmo2239*	*lmo2241 lmo2240 lmo2239*	70
lmo2372	*lmo2371*	*lmo2371 lmo2372*	
lmo2745[c]		monocistronic	50
lmo2751[c]		*lmo2751 lmo2752*	38
lmo2752[c]			46
eslA	*eslB*	*lmo2769 (eslA) lmo2768 (eslB) lmo2767 (eslC) lmo2766 (eslR)*	30

[a]Operons according to Toledo-Arana et al. (2009), possible transcriptional regulators are underlined.

[b]TPM—transcripts per million as an estimate for gene expression according to previously published RNA-Seq data of *L. monocytogenes* wild-type strain EGD-e during exponential growth, TPM values for the strictly repressed *lieAB* genes were ~10 (Hauf et al., 2019).

[c]Contains both an ATPase and a permease domain in one polypeptide.

[d]Not detected by Toledo-Arana et al. (2009), but genetic arrangement suggests organization as an operon.

Interestingly, some of the transporter genes are arranged in operons together with genes that are annotated as genes encoding GntR-, MarR-, MngR-, LytR- or TetR-like transcriptional regulators (Table 1), suggesting transcriptional induction during specific conditions. Most likely, these transcriptional regulators respond to low molecular weight ligands such as metabolic intermediates or toxic compounds including antibiotics (Gupta et al., 2018; Housseini et al., 2018; Jain, 2015). None of these 23 ABC transporters is associated with an extracellular substrate-binding protein, further suggesting that they all may act as compound exporters.

In order to identify conditions that lead to transcriptional induction of these uncharacterized MDR transporter genes or operons, we fused their 18 promoters to *lacZ* and inserted these promoter-*lacZ* fusions into the chromosome of strain EGD-e. These strains were then grown in BHI broth at 37°C to mid-logarithmic growth phase and β-galactosidase activity was determined. Promoter activity ranged between 14 ± 3 miller units (MU) for $P_{lmo0741}$ and 356 ± 64 MU for $P_{lmo2215}$ (Figure 1). Median activity of the 18 tested promoters was 67 MU, which compares to the low activity of the P_{lieAB} promoter when repressed by LftR (45 ± 8 MU) and which is only somewhat higher than seen in a strain carrying the promoterless *lacZ* gene (13 ± 3 MU). In contrast, relief of LftR repression of the P_{lieAB} promoter in a Δ*lftR* background leads to a β-galactosidase activity as high as 8513 ± 1508 MU (Figure 1), which is in good agreement with previous results (Hauf et al., 2019, 2021). This shows that most of the MDR transporter genes are poorly expressed under standard growth conditions.

2.2 | Tartrolons induce expression of the *tim* operon

A natural compound collection was used to identify substances that induce the expression of the 18 ABC transporter gene

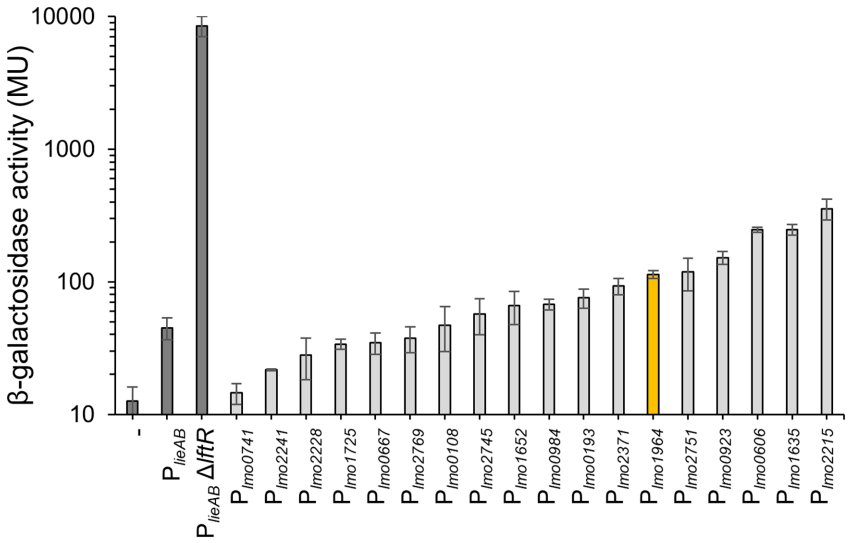

FIGURE 1 Promoters driving expression of potential MDR ABC transporter genes in *L. monocyctogenes*. Background activity of MDR ABC transporter gene promoters (light gray). Measurements of β-galactosidase activity in *L. monocytogenes* strains expressing promoter-*lacZ* fusions. Measurements were performed on strains grown in BHI broth at 37°C until midlogarithmic growth phase. Average values and standard deviations were calculated from three independent repetitions. Strains carrying a P_{lieAB} promoter *lacZ* fusion either in wild type (LMSH5) or the Δ*lftR* background (LMSH98) or a promoter-less *lacZ* gene (LMSH16, labeled with a minus sign) were used as controls (dark gray). The promoter of the *timABR* (*lmo1964-lmo1962*) operon is highlighted in orange.

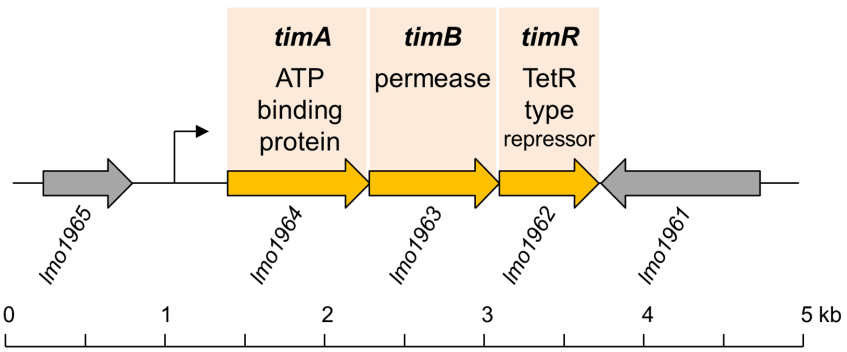

FIGURE 2 Schematic illustration of the *timABR* ABC transporter locus of *L. monocytogenes* EGD-e.

promoters in a drop diffusion assay. This set as part of the DZIF (German Centre for Infection Research) natural product collection (DZIF/TTU9, 2023) contained 681 secondary metabolites purified from myxobacteria (253), streptomycetes (340) and fungi (88). The activity of the promoter of the *lmo1964-lmo1963-lmo1962* operon (Figure 2) was found to be induced by tartrolons (Figure 3a). This operon (Toledo-Arana et al., 2009) encodes the ATPase (*lmo1964*) and permease (*lmo1963*) subunits of an uncharacterized ABC transporter and a gene encoding a TetR-type transcriptional repressor (*lmo1962*, Figure 2).

For confirmation, promoter induction was tested with tartrolon A and B preparations, whose purity was separately validated using liquid chromatography-high resolution mass spectrometry (LC-hrMS, Figure S1). Induction was observed with tartrolon B and—to a lesser extent—with tartrolon A as concluded from the appearance of a blue ring that surrounds the compound application site in the agar-based diffusion assay with strain LMTE19 (*attB*::$P_{lmo1964}$-*lacZ*) as the reporter strain. Both compounds generated zones of growth inhibition indicating toxicity, but while tartrolon A only caused promoter induction at the edge of the growth inhibition zone, tartrolon B activated the *lmo1964* promoter at concentrations that did not prevent growth (Figure 3a). The only other hit was the induction of the promoter controlling the expression of the *lmo0193-lmo0194-lmo0195* operon by gallidermin, a lantibiotic produced by *Staphylococcus gallinarum* (Kellner et al., 1988) and to a lesser extent by enduracidin, a polypeptide antibiotic synthesized by *Streptomyces fungicidicus* (Yin & Zabriskie, 2006).

Tartrolons A and B are macrodiolide antibiotics isolated from *Sorangium cellulosum*, a soil-dwelling myxobacterium (Irschik et al., 1995; Schummer et al., 1994). They consist of a 42-membered macrocyclic ring with four free hydroxyl groups in tartrolon A. In tartrolon B, however, these hydroxyl group oxygens are covalently bound to a single boron atom (Schummer et al., 1994) (Figure 3b). Tartrolons inhibit growth of different Gram-positive bacteria and block DNA, RNA and protein biosynthesis of *Staphylococcus aureus* (Irschik et al., 1995). Tartrolons are structurally similar to boromycin

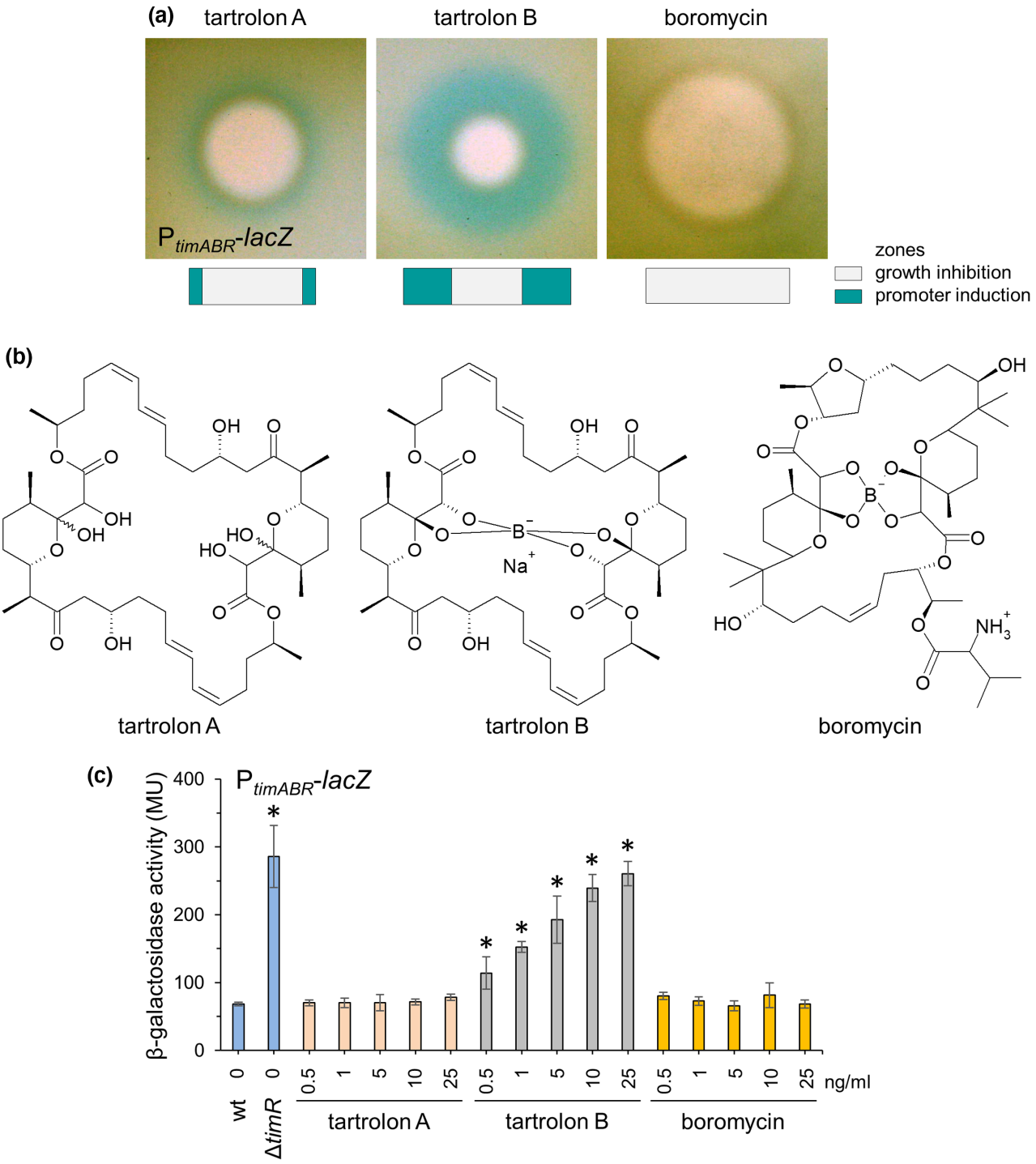

FIGURE 3 Induction of the P_{timABR} promoter by tartrolons A and B and boromycin. (a) Induction of the P_{timABR} promoter in an agar-based assay. Strain LMTE19 (P_{timABR}-*lacZ*) was included in X-Gal containing BHI agar, and 2 μL of compound solutions (2.5 μg/μL each) was spotted on top. The images were taken after overnight incubation at 37°C. The drawings below mark the zones of growth inhibition and promoter induction. (b) Chemical structures of tartrolon A, tartrolon B and boromycin. (c) Concentration-dependent induction of the P_{timABR} promoter by tartrolons and boromycin. LMTE19 (P_{timABR}-*lacZ*) was grown in BHI broth containing increasing compound concentrations to mid-logarithmic growth phase at 25°C, and β-galactosidase activity was determined. Strain LMTE50 (Δ*timR* P_{timABR}-*lacZ*) was included for comparison. Average values and standard deviations were calculated from three independent repetitions. Asterisks mark statistically significant differences compared to LMTE19 without tartrolon (labeled "wt," $p < 0.05$, *t*-test with Bonferroni-Holm correction).

(Figure 3b), another boron containing macrocyclic antibiotic (Hütter et al., 1967), however, boromycin did not induce the $P_{lmo1964}$ promoter (Figure 3a).

To further support the finding of tartrolon-specific induction of the $P_{lmo1964}$ promoter, β-galactosidase activity was determined in strain LMTE19 in the presence of increasing compound

concentrations. While no induction was observed with tartrolon A and boromycin up to non-inhibitory compound concentrations of 25 ng/mL, tartrolon B induced this promoter already at a concentration of 0.5 ng/mL and promoter activity further increased in a concentration-dependent manner almost to the same degree as observed when the *lmo1962* repressor gene was deleted (Figure 3c). Thus, tartrolon B is a specific inducer of the *L. monocytogenes* $P_{lmo1964}$ promoter and the protein encoded by the *lmo1962* gene functions as a transcriptional repressor of its own operon.

2.3 | The *timABR* genes confer tartrolon resistance

Induction of $P_{lmo1964}$ by growth-inhibiting tartrolons raised the possibility that the potential MDR transporter encoded by the *lmo1964-lmo1963* genes could mediate resistance of *L. monocytogenes* against tartrolons. To test this hypothesis, a deletion mutant lacking the *lmo1964-lmo1963* genes was generated. Resistance of this mutant against tartrolon B was almost 200-fold reduced (MIC: 0.009 ± 0.005 μg/mL) compared to wild type (MIC: 1.7 ± 0.6 μg/mL), and a similar, but less pronounced effect was observed for tartrolon A (Figure 4a). Reintroduction of an IPTG-inducible copy of the *lmo1964-lmo1963* genes into the Δ*lmo1964-lmo1963* mutant generated a strain that showed tartrolon A and B resistance similar to the Δ*lmo1964-lmo1963* mutant in the absence of IPTG and wildtype-like tartrolon resistance when IPTG was present (Figure 4a). In agreement with its function as a repressor, deletion of *lmo1962* further increased tartrolon B resistance (MIC: 4 ± 0 μg/mL) and reintroduction of the gene complemented this effect. In contrast, resistance against tartrolon A was not affected by the *lmo1962* deletion (Figure 4a). Since the *lmo1964-lmo1962* operon can be induced by tartrolons and confers tartrolon resistance, we propose to rename these three genes as *timA* (*lmo1964*), *timB* (*lmo1963*) and *timR* (*lmo1962*, tartrolon inducible MDR transporter).

We also determined the resistance of the Δ*timAB* mutant against selected antibiotics (β-lactams, ciprofloxacin, vancomycin, erythromycin, tetracycline, bacitracin, chloramphenicol, fosfomycin, rifampicin, ADEP), dyes (ethidium bromide, rhodamine 6G) and benzalkonium chloride as a biocide, in order to find out whether TimAB would also mediate resistance against other known MDR transporter substrates. However, the susceptibility against none of the tested substances was altered in the absence of the TimAB transporter (Figure S2a,b).

Next, we asked whether the *timAB* genes would be sufficient to mediate protection against tartrolon antibiotics. To address this, the *L. monocytogenes timAB* genes were heterologously expressed in *B. subtilis* from a xylose-inducible promoter. The MICs of tartrolon A

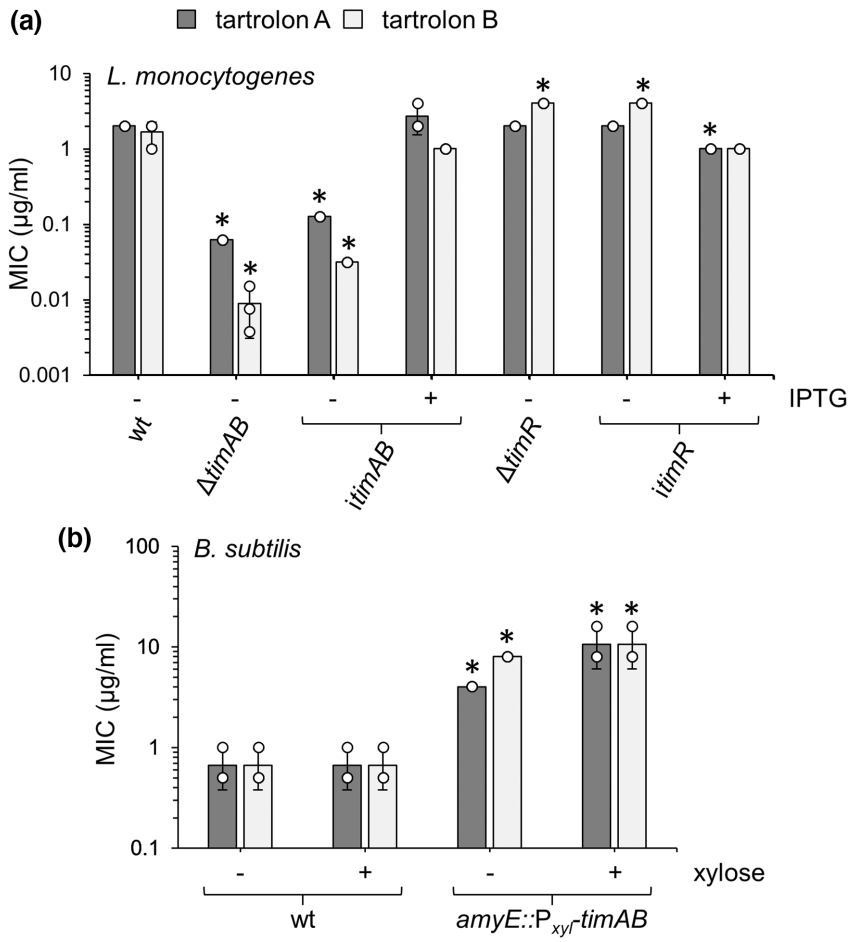

FIGURE 4 Contribution of *timABR* genes to tartrolon resistance. (a) Effect of *timAB* genes on resistance against tartrolon A and B. Minimal inhibitory concentrations (MICs) of *L. monocytogenes* strains EGD-e (wt), LMTE34 (Δ*timAB*), LMTE37 (Δ*timR*), LMTE51 (i*timAB*) and LMTE52 (i*timR*) grown in BHI broth ±1 mM IPTG were determined in broth dilution assays. (b) Effect of *timAB* expression on tartrolon resistance of *B. subtilis*. MICs of tartrolon A and B were determined for *B. subtilis* strains 168 (wt) and BSTE1 (*amyE*::P_{xyl}-*timAB*) grown in LB broth ±0.5% xylose. Average values and standard deviations were calculated from three independent repetitions and individual data points are also shown. Asterisks mark statistically significant differences compared to wild type ($p < 0.05$, *t*-test with Bonferroni-Holm correction). Raw data of the experiments shown in both panels are given in Table S1.

and B were 0.7 ± 0.3 μg/mL for the *B. subtilis* wild-type strain 168 in each case. In contrast, strain BSTE1 (*amyE*::P_{xyl}-*timAB*) had approximately ten-fold higher MICs for tartrolon A (4 ± 0 μg/mL) and tartrolon B (8 ± 0 μg/mL) and the MICs for both compounds increased even to 10.7 ± 4.4 μg/mL in the presence of xylose (Figure 4b). In contrast to this, resistance of *B. subtilis* to boromycin (MIC for wild type: 1.33 ± 0.58 μg/mL) was not affected by transplantation and expression of the *timAB* genes (the MIC for strain BSTE1 without xylose was 1.33 ± 0.58 μg/mL and with xylose 1.67 ± 0.58 μg/mL). This shows that the *timAB* genes encode a novel transporter required, sufficient and specific for resistance against tartrolons.

2.4 | TimR binding to the P_{timABR} promoter is sensitive to tartrolon B

Deletion of *timR* resulted in a four-fold increase of P_{timABR} promoter activity (Figure 3c) and also increased tartrolon B resistance (Figure 4a), which suggested that TimR could repress transcription of its own operon. To further test this hypothesis, TimR was purified as a Strep-tagged protein (Figure S3a) and binding of TimR to the P_{timABR} promoter fragment was analyzed in an electrophoretic mobility shift assay (EMSA). As can be seen in Figure 5a, addition of TimR retarded the P_{timABR} promoter fragment in the gel and two complexes appeared instead, out of which the slower migrating species remained when the TimR concentration was further increased. Most likely, TimR is dimeric as known for other TetR-type transcriptional repressors (Bertram et al., 2021) and increasing concentrations favor TimR dimerization. The interaction of TimR with the *timABR* promoter was specific as a *divIVA* promoter fragment was not retarded (Figure 5a). Intriguingly, no complex formation between TimR and its promoter was observed when the same experiment was repeated in the presence of tartrolon B, indicating that tartrolon B prevents binding of TimR to the P_{timABR} promoter fragment (Figure 5b). In contrast, promoter binding of TimR was neither prevented by tartrolon A or boromycin (Figure S3b), demonstrating that promoter binding by TimR is specifically sensitive to tartrolon B.

The DNA fragment tested for TimR binding covered the 450 bp in front of the *timA* start codon. BPROM (Solovyev & Salamov, 2011) detected a possible promoter sequence in this region and the identified promoter was congruent with a previously identified transcription start site (Wurtzel et al., 2012). Several inverted repeats that potentially could serve as binding sites for TimR were also found, but not further tested (Figure S4).

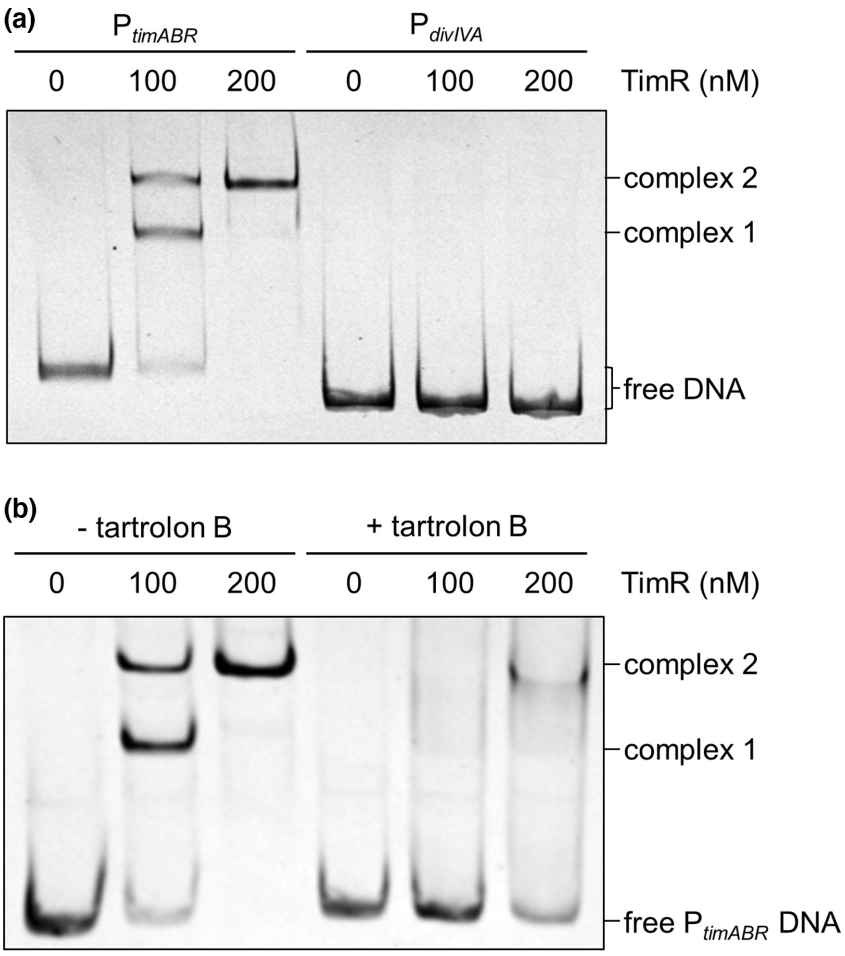

FIGURE 5 TimR binding to the P_{timABR} promoter is sensitive to tartrolon B. (a) Electrophoretic mobility shift assay (EMSA) showing the interaction of TimR-Strep with a P_{timABR} promoter fragment. The promoter of the *divIVA* gene was chosen to demonstrate specificity. (b) EMSA showing the effect of tartrolon B on the interaction of TimR-Strep with the P_{timABR} promoter. The binding assay was carried out in the absence and the presence of 1.25 μg/mL tartrolon B. The positions of unbound DNA and the two TimR-promoter nucleoprotein complexes are indicated.

2.5 | Evidence for the mode of action of tartrolon B as potassium ionophore

Boromycin acts as a potassium ionophore, causing leakage of potassium ions out of the cell (Moreira et al., 2016; Pache & Zähner, 1969). This explains boromycin toxicity, as high intracellular potassium concentrations are critical for pH homeostasis in the cytosol, maintenance of turgor and membrane potential as well as for ribosome activity (Epstein, 2003; Gundlach et al., 2017). We wondered whether the structurally related tartrolon B would act in a similar way, and for this, we asked whether external potassium supply would suppress efficacy of tartrolon B. As can be seen in Figure 6, a 200-fold increase of the MIC of boromycin was observed, when 250 mM potassium chloride was added to the growth medium. This is in good agreement with boromycin acting as a potassium ionophore and is consistent with other reports (Moreira et al., 2016, Pache & Zähner, 1969). Importantly, a similar effect was also observed for tartrolon B, whose MIC increased 32-fold in the presence of potassium chloride. In contrast, addition of 250 mM sodium chloride did not result in a similar effect and rather yielded a slight sensitization of *L. monocytogenes* against both antibiotics. This shows that externally supplied potassium ions specifically suppress the toxicity of boromycin and tartrolon B against *L. monocytogenes* and this would be in good agreement with a potassium ionophore mode of action.

2.6 | Tartrolon B resistance of other *Listeria* species

The *timABR* genes are part of the *L. monocytogenes* core genome (Ruppitsch et al., 2015), and they are present in all closed *L. monocytogenes* genomes currently available (data not shown). Likewise, *timABR* homologs are found in all other *Listeria* sensu stricto species (*L. innocua*, *L. ivanovii*, *L. marthii*, *L. seeligeri* and *L. welshimeri*) and in the *Listeria* species belonging to the *Paenilisteria* clade (Orsi & Wiedmann, 2016), while the *timABR* gene cluster was found to be absent from the genomes of *Listeria* species belonging to the *Mesolisteria* and *Murraya* groups (Figure S5). Moreover, the *timR* gene of *Listeria cornellensis* FSL F6-0969 and the *timB* gene of *Listeria riparia* FSL S10-1204 are truncated (Figure S5) (den Bakker et al., 2014), suggesting that selection for loss or increase of tartrolon resistance is still ongoing or might have occurred parallel to speciation.

We measured tartrolon B resistance of selected *Listeria* sensu stricto (*timABR* positive), *Mesolisteria* (*timABR* negative) and *Murraya* species (*timABR* negative) and found that tartrolon B resistance levels were comparable to *L. monocytogenes* among the three other tested *Listeria* sensu stricto *species* (Table 2) that all contained *timABR* (Figure S5). Unexpectedly, tartrolon B resistance of *Mesolisteria* species was found to be similar (*L. floridensis*) or even higher (*L. aquatica* and *L. fleischmannii*) as in *L. monocytogenes* (Table 2), even though *timABR* was absent (Figure S5). In contrast, *L. grayi*,

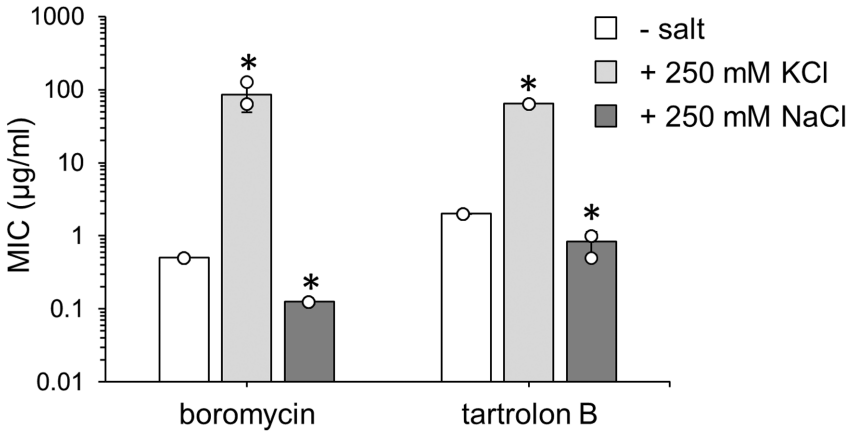

FIGURE 6 Tartrolon B action is potassium-sensitive. MICs of tartrolon B for *L. monocytogenes* EGD-e in the absence and presence of 250 mM KCl. Boromycin (positive control) and NaCl (negative control) were included for comparison. MICs were determined three times. Average values with standard deviations and individual data points are shown. Asterisks mark statistically significant differences (*t*-test with Bonferroni-Holm correction, $p < 0.05$). Raw data are given in Table S2.

TABLE 2 Resistance of selected *Listeria* species against tartrolon B.

Species	Group	*timABR*	MIC tartrolon B (µg mL^{-1})[a]
L. monocytogenes	*Listeria* sensu stricto	+	1.3 ± 0.6
L. innocua	*Listeria* sensu stricto	+	6.6 ± 2.3
L. ivanovii	*Listeria* sensu stricto	+	0.7 ± 0.3
L. seeligeri	*Listeria* sensu stricto	+	1.3 ± 0.6
L. aquatica	*Mesolisteria*	−	>16
L. fleischmannii	*Mesolisteria*	−	>16
L. floridensis	*Mesolisteria*	−	2.3 ± 1.5
L. grayi	*Murraya*	−	0.004 ± 0.003

[a]Average values and standard deviations were calculated from three independent experiments.

representing the *Murraya* clade, was as sensitive to tartrolon B as the *L. monocytogenes* Δ*timAB* mutant (Table 2, Figure 4a). The absence of *timABR* from the *L. grayi* genome would explain this sensitivity, but *timABR*-independent tartrolon B resistance mechanisms must have evolved in *Mesolisteria*.

A cblaster search was performed to identify non-*Listeria* species that carry the complete *timABR* gene cluster. This algorithm is designed to detect homologs of gene clusters (Gilchrist et al., 2021). Cblaster confirmed the presence of the *timABR* cluster in *Listeria* sensu stricto and *Paenilisteria* species and its absence in the *Mesolisteria* and *Murraya*. Outside the *Listeriaceae*, *timABR* homologous clusters were identified in several *Enterococcaceae*, *Bacillaceae*, *Sporolactobacillaceae* and *Paenibacillaceae* as well as in few species beyond the *Bacilli* (Figure S6).

3 | DISCUSSION

As a ubiquitously occurring environmental bacterium, *L. monocytogenes* has developed manifold strategies to survive the conditions prevailing in its multifaceted habitats. The stress response mechanisms of *L. monocytogenes* facing those harmful conditions that are most obviously occurring during life in the environment such as heat/cold, high osmolarity or exposure to ultraviolet light are generally well understood (Bucur et al., 2018; Lakicevic et al., 2021; Quereda et al., 2021). The response to conditions specific to the passage through the gastrointestinal tract including the extreme acidity in the stomach or the presence of bile salts in the gut has also been investigated (Arcari et al., 2020; Gahan & Hill, 2014). In contrast, studies on how *L. monocytogenes* interacts with or outcompetes other species of the gut microbiota by production of bacteriocins such as listeriolysin S or Lmo2776 began to move into the focus of research just recently (Hafner et al., 2021; Quereda et al., 2017; Rolhion et al., 2019), even though research on listerial resistance against such bacteriocins, particularly nisin, which is used as a food preservative, has a long tradition (Kaur et al., 2011). Despite intensive investigations of the confrontation of *L. monocytogenes* with this wide spectrum of noxious conditions/agents, only few export systems are known that are used by *L. monocytogenes* to withstand antibiotics produced by rivaling species in habitats outside the human host. Among these are transporters for the export of bacitracin (AnrAB), nisin (AnrAB, VirAB), kanamycin and tetracycline (VirAB) and macrolides (MdrL) (Collins et al., 2010; Grubaugh et al., 2018; Jiang et al., 2019; Mata et al., 2000). In addition to MdrL, *L. monocytogenes* encodes several further MFS type transporters; however, as far as currently known they contribute to interferon induction during infection by extrusion of cyclic-di-AMP (Crimmins et al., 2008; Kaplan Zeevi et al., 2013; Woodward et al., 2010).

One recently identified example for systems providing resistance against antibiotics produced by natural competitors is the LftRS/LieAB system enabling *L. monocytogenes* to sense and to detoxify aurantimycin A (Hauf et al., 2019, 2021), which is produced by *S. aurantiacus*, another inhabitant of the soil (Gräfe et al., 1995). With the TimABR system, we here add another example to the list of *L. monocytogenes* systems providing resistance against an antibiotic synthesized by a soil-dwelling microorganism co-residing in the same habitat. Similar to the LftRS/LieAB system, this system is composed of an ABC transporter for compound export and a transcriptional regulator for compound sensing.

While direct sensing of aurantimycin by its cognate transcriptional regulator LftR has never been shown (Hauf et al., 2019, 2021), a direct interaction of tartrolon B with TimR, the transcriptional regulator of the TimABR system, must be concluded from the observation that tartrolon B addition prevents promoter binding of TimR. Remarkably, this interaction was specific to the borate-containing tartrolon B and could not be observed with the identical but borate-free macrodiolide ring present in tartrolon A. The conformation of the macrocycle in tartrolon B and thus its ability to bind to TimR might be altered after borate esterification. Alternatively, TimR could make direct contacts with the borate ester part of the tartrolon B molecule through electrostatic interactions or could form hydrogen bonds with the borate oxygens as observed in *Vibrio harveyi* LuxP, the sensor of the borate diester containing quorum sensing autoinducer-2 (Chen et al., 2002). Interestingly, even covalent bonds between boron atoms of organoboron compounds and amino acid side chains of proteins have been reported (Newman et al., 2021; Nguyen et al., 2022) and could potentially be involved in tartrolon B recognition by TimR.

Despite this remarkable specificity of TimR for tartrolon B, tartrolon detoxification by TimAB is not sensitive to the presence of the borate ester, as judged from the *timAB* transplantation experiment into *B. subtilis*, where the MICs of tartrolon A and tartrolon B increased to exactly the same level after induction of *timAB* expression. However, the MIC of tartrolon B for the *L. monocytogenes* Δ*timAB* mutant is approximately seven-fold lower than that of tartrolon A, indicating that tartrolon B is more toxic to *L. monocytogenes* in the absence of a suitable export mechanism. As the MICs for tartrolon A and tartrolon B do not differ significantly in the *L. monocytogenes* wild-type background, though, we have to assume that tartrolon B is being exported at a higher rate than tartrolon A in the presence of the *timABR* cassette. This latter conclusion is in good agreement with the observation that the *timABR* promoter is stronger activated by tartrolon B as compared to tartrolon A.

Tartrolons were identified in the soil-dwelling myxobacterium *S. cellulosum* (Irschik et al., 1995; Schummer et al., 1994), from a *Streptomyces* isolate found in a marine sediment (Perez et al., 2009) and from *Teredinibacter turnerae*, a γ-proteobacterium isolated from the gills of marine shipworms (Elshahawi et al., 2013). *L. monocytogenes* had frequently been isolated from soil samples of various origin (Vivant et al., 2013), its isolation from seawater and estuarine water samples was reported at least occasionally (Bou-m'handi et al., 2007; El-Shenawy & El-Shenawy, 2006; Rodas-Suarez et al., 2006) and very often the pathogen is found on seafood products (Dillon & Patel, 1992; Lachmann et al., 2022). *L. monocytogenes* tolerates high salinity (Nolan et al., 1992; Shahamat et al., 1980), and therefore, isolation from marine environments seems plausible. The strong

conservation of the *timABR* locus in selected *Listeria* lineages could reflect a tight ecological interaction between tartrolon-producing microorganisms present in the soil and in marine environments and such *timABR*-positive *Listeria* species.

The *timABR* cassette is only one out of the 18 ABC transporter operons with which we started this project. The inducing agents and/or the compounds exported by the remaining ones are still unknown. We assume that the specific compilation of these still-to-be-characterized ABC transporters represents a genetic signature that reflects the ecological interactions that *L. monocytogenes* experiences with its most important antibiotic-producing competitors in nature. Understanding these interactions in combination with knowledge on the prevalence of the compound producers in nature might help to better specify the environmental reservoir of *L. monocytogenes*.

4 | EXPERIMENTAL PROCEUDRES

4.1 | Bacterial strains and growth conditions

All strains and plasmids used in this study are listed in Table 3. Non-*L. monocytogenes Listeria* strains were received from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig). *Listeria* strains were grown in BHI broth or on BHI agar plates at 37°C. Strains of *B. subtilis* were cultivated in LB broth or on LB agar plates. Erythromycin (5 μg mL^{-1}), kanamycin (50 μg mL^{-1}), spectinomycin (50 μg mL^{-1}), X-Gal (100 μg mL^{-1}) or IPTG (1 mM) were added as indicated where required. *Escherichia coli* TOP10 was used as the standard cloning host (Sambrook et al., 1989). Tartrolon A and B were obtained from the DZIF natural compound selection, and boromycin was purchased from Hello Bio Ltd. (Ireland).

4.2 | General methods, manipulation of DNA and oligonucleotide primers

Standard methods were used for transformation of *E. coli* and for isolation of plasmid DNA (Sambrook et al., 1989). Transformation of *L. monocytogenes* and *B. subtilis* was carried out as described by others (Hamoen et al., 2002; Monk et al., 2008). Restriction and ligation of DNA was performed following the manufacturer's instructions. All primer sequences are listed in Table S3.

4.3 | Construction of bacterial plasmids and strains

Plasmids carrying *lacZ* fusions to the promoters of ABC transporter operons were constructed by amplification of the promotor sequences using primers TE45/TE46 ($P_{lmo0606}$), TE47/TE48 ($P_{lmo0741}$), TE49/TE50 ($P_{lmo0923}$), TE51/TE52 ($P_{lmo1635}$), TE53/TE54 ($P_{lmo2215}$), TE66/TE67 ($P_{lmo0984}$), TE68/TE69 ($P_{lmo0108}$), TE70/TE71 ($P_{lmo1652}$), TE72/TE181 ($P_{lmo1725}$), TE74/TE75 (P_{timABR}), TE76/TE77 ($P_{lmo2228}$), TE78/TE79 ($P_{lmo2241}$), TE80/TE81 ($P_{lmo2371}$), TE82/TE83 ($P_{lmo2745}$), TE86/TE87

TABLE 3 Plasmids and strains used in this study.

Name	Relevant characteristics	Source/Reference[a]
Plasmids		
pBP117	*lacZ neo*	Hauf et al. (2019)
pET11a	*bla* P_{T7} *lacI*	Novagen
pIMK3	P_{help}-*lacO lacI neo*	Monk et al. (2008)
pMAD	*bla erm bgaB*	Arnaud et al. (2004)
pSG1154	*bla amyE3′ spc* P_{xyl} *amyE5′*	Lewis and Marston (1999)
pTE6	$P_{lmo0923}$-*lacZ neo*	This work
pTE7	$P_{lmo1635}$-*lacZ neo*	This work
pTE8	$P_{lmo2215}$-*lacZ neo*	This work
pTE10	$P_{lmo0741}$-*lacZ neo*	This work
pTE12	$P_{lmo0108}$-*lacZ neo*	This work
pTE14	$P_{lmo0984}$-*lacZ neo*	This work
pTE15	$P_{lmo1652}$-*lacZ neo*	This work
pTE16	P_{timABR}-*lacZ neo*	This work
pTE17	$P_{lmo2228}$-*lacZ neo*	This work
pTE19	$P_{lmo2241}$-*lacZ neo*	This work
pTE23	$P_{lmo0606}$-*lacZ neo*	This work
pTE24	$P_{lmo2751}$-*lacZ neo*	This work
pTE26	$P_{lmo2371}$-*lacZ neo*	This work
pTE32	$P_{lmo2745}$-*lacZ neo*	This work
pTE36	$P_{lmo2769}$-*lacZ neo*	This work
pTE37	$P_{lmo1725}$-*lacZ neo*	This work
pTE39	$P_{lmo0667}$-*lacZ neo*	This work
pTE42	*bla erm bgaB* Δ*timAB* (*lmo1964-lmo1963*)	This work
pTE52	*bla erm bgaB* Δ*timR* (*lmo1962*)	This work
pTE58	*bla amyE3′ spc* P_{xyl}-*timAB amyE5′*	This work
pTE61	P_{help}-*lacO-timAB lacI neo*	This work
pTE62	P_{help}-*lacO-timR lacI neo*	This work
pTE71	*bla* P_{T7}-*timR-strep lacI*	This work
pTE75	$P_{lmo0193}$-*lacZ neo*	This work
B. subtilis strains		
168	Wild type	Lab stock
BSTE1	*amyE*::P_{xyl}-*timAB spc*	pTE58 → 168
L. monocytogenes strains		
EGD-e	Wild type	Glaser et al. (2001)
LMSH5	*attB*::$P_{lieAB(1-266)}$-*lacZ neo*	Hauf et al. (2021)
LMSH16	*attB*::*lacZ neo*	Hauf et al. (2019)
LMSH98	Δ*lftR attB*::$P_{lieAB(1-122)}$-*lacZ neo*	Hauf et al. (2021)
LMTE3	*attB*::$P_{lmo0741}$-*lacZ neo*	pTE10 → EGD-e
LMTE4	*attB*::$P_{lmo0923}$-*lacZ neo*	pTE6 → EGD-e

TABLE 3 (Continued)

Name	Relevant characteristics	Source/Reference[a]
LMTE5	*attB*::$P_{lmo1635}$-*lacZ neo*	pTE7 → EGD-e
LMTE6	*attB*::$P_{lmo2215}$-*lacZ neo*	pTE8 → EGD-e
LMTE10	*attB*::$P_{lmo2241}$-*lacZ neo*	pTE19 → EGD-e
LMTE11	*attB*::$P_{lmo0984}$-*lacZ neo*	pTE14 → EGD-e
LMTE12	*attB*::$P_{lmo2228}$-*lacZ neo*	pTE17 → EGD-e
LMTE14	*attB*::$P_{lmo1652}$-*lacZ neo*	pTE15 → EGD-e
LMTE15	*attB*::$P_{lmo0108}$-*lacZ neo*	pTE12 → EGD-e
LMTE16	*attB*::$P_{lmo2751}$-*lacZ neo*	pTE24 → EGD-e
LMTE18	*attB*::$P_{lmo2371}$-*lacZ neo*	pTE26 → EGD-e
LMTE19	*attB*::P_{timABR}-*lacZ neo*	pTE16 → EGD-e
LMTE24	*attB*::$P_{lmo2769}$-*lacZ neo*	pTE36 → EGD-e
LMTE26	*attB*::$P_{lmo2745}$-*lacZ neo*	pTE32 → EGD-e
LMTE27	*attB*::$P_{lmo0606}$-*lacZ neo*	pTE23 → EGD-e
LMTE28	*attB*::$P_{lmo0667}$-*lacZ neo*	pTE39 → EGD-e
LMTE33	*attB*::$P_{lmo1725}$-*lmo1725-lacZ neo*	pTE37 → EGD-e
LMTE34	Δ*timAB*	pTE42 ↔ EGD-e
LMTE37	Δ*timR*	pTE52 ↔ EGD-e
LMTE50	Δ*timR attB*::P_{timABR}-*lacZ neo*	pTE16 → LMTE37
LMTE51	Δ*timAB attB*::P_{help}-*lacO-timAB lacI neo*	pTE61 → LMTE34
LMTE52	Δ*timR attB*::P_{help}-*lacO-timR lacI neo*	pTE62 → LMTE37
LMTE71	*attB*::$P_{lmo0193}$-*lacZ neo*	pTE75 → EGD-e
Listeria species		
L. innocua DSM 20649		DSMZ (Braunschweig, Germany)
L. ivanovii subsp. ivanovii DSM 20750		DSMZ (Braunschweig, Germany)
L. seeligeri DSM 20751		DSMZ (Braunschweig, Germany)
L. aquatica DSM 26686		DSMZ (Braunschweig, Germany)
L. fleischmannii subsp. fleischmannii DSM 24998		DSMZ (Braunschweig, Germany)
L. floridensis DSM 26687		DSMZ (Braunschweig, Germany)
L. grayi DSM 20596		DSMZ (Braunschweig, Germany)

[a]The arrow (→) stands for a transformation event, and the double arrow (↔) indicates gene deletions obtained by chromosomal insertion and subsequent excision of pMAD plasmid derivatives (see experimental procedures for details).

($P_{lmo2751}$), TE88/TE89 ($P_{lmo2769}$), TE124/TE125 ($P_{lmo0667}$) or TE277/TE278 ($P_{lmo0193}$). The resulting fragments were inserted into pBP117 using restriction-free (RF) cloning (van den Ent & Löwe, 2006).

For construction of plasmid pTE42, allowing deletion of *timAB*, fragments up- and downstream to *timAB* were amplified using oligonucleotides TE135/TE136 and TE137/TE134, respectively. Both fragments were then fused together by splicing by overlapping extension (SOE)-PCR and introduced into pMAD by RF cloning. Plasmid pTE52, designed for deletion of *timR*, was obtained by amplification of *timR* up- and downstream fragments using the primer pairs TE213/TE214 and TE215/TE212, respectively. These fragments were spliced together by SOE-PCR, and the resulting fragment was introduced into pMAD by RF cloning.

Plasmid pTE61 was constructed for inducible *timAB* expression. It was obtained by amplification of *timAB* using the oligonucleotides TE227/TE228 and cloning of the resulting fragment into pIMK3 using NcoI/SalI.

Plasmid pTE62 was constructed for IPTG-dependent *timR* expression. To this end, the *timR* gene was amplified using TE229/TE230 as the primers and inserted into pIMK3 using NcoI/SalI.

Plasmid pTE71 was generated for purification of strep-tagged TimR. For this, the *timR* gene was amplified with oligonucleotides TE239/TE240 and integrated into pET11a through RF cloning.

Plasmid pTE58 was constructed for heterologous *timAB* expression in *B. subtilis*. To this end, the *timAB* genes were amplified using the primer pair TE218/TE219, and the resulting fragment was cloned into pSG1154 using RF cloning.

Derivatives of pIMK3 and pBP117 plasmids were introduced into *L. monocytogenes* strains by electroporation and transformants were selected on BHI agar plates containing kanamycin at 37°C. Plasmid integration at the $tRNA^{Arg}$ *attB* site was confirmed by PCR. Likewise, plasmid derivatives of pMAD were introduced into *L. monocytogenes*, but transformants were selected on BHI agar plates containing X-Gal and erythromycin at 30°C. The plasmid integration-excision protocol described by Arnaud et al. (2004) was then used for gene deletions. All gene deletions were confirmed by PCR. *B. subtilis* was transformed with plasmid pTE58 and transformants were selected on LB agar plates containing spectinomycin (50 μg/mL). Integration of the plasmid into the *amyE* locus was confirmed by absence of amylase activity on starch containing agar plates.

4.4 | Natural compound screen

Natural compounds were compiled by the German Centre for Infection Research (DZIF) infrastructure and provided as "Natural Compound Library" through the Helmholtz Institute for Pharmaceutical Research Saarland (HIPS) as a ready-to-screen library (DZIF/TTU9, 2023). The compilation includes 681 purified secondary metabolites from myxobacteria (253), fungi (88) and streptomycetes (340), collected in natural product screening programs at HIPS, the Helmholtz Centre for Infection Research (HZI), and the University of Tübingen, respectively.

Library compounds were provided as 1 mM stock solutions in DMSO in conical 96-well plates in a randomized order and encrypted by a barcode system for non-biased screening. For the screen, *L. monocytogenes* reporter strains were grown overnight in BHI broth at 37°C and diluted 1:2000 in molten BHI agar containing 50 μg/mL X-Gal, which was cooled down to ~50°C prior to mixing and plate pouring. Compounds were applied on top of the agar plates (1 μL each) at a concentration of 0.033 mM (in DMSO) and plates were incubated overnight at 37°C.

4.5 | Compound quality control

Compounds included in the natural product library are routinely checked by HPLC-MS and NMR. Here, we additionally analyzed the batches of tartrolons A and B that were used for extended biological assays. Measurements were performed with a Dionex Ultimate 3000 RSLC system (Thermo) using a BEH C18, 100 × 2.1 mm, 1.7 μm dp column (Waters). Separation of 1 μL sample was achieved by a linear gradient from (A) H_2O + 0.1% FA to (B) ACN + 0.1% FA at a flow rate of 600 μL/min and 45°C. The gradient was initiated by a 0.5 min isocratic step at 5% B, followed by an increase to 95% B in 18 min to end with a 2 min step at 95% B before re-equilibration with initial conditions. UV spectra were recorded by a diode array detector (DAD) in the range from 200 to 600 nm. The LC flow was split to 75 μL/min before entering the timsTOF fleX mass spectrometer (Bruker Daltonics). The split was set up with fused silica capillaries of 75 and 100 μm I.D. and a low dead volume tee junction (Upchurch). The timsTOF fleX was operated in positive ESI mode, with 1.0 bar nebulizer pressure, 5.0 L/min dry gas, 200°C dry heater, 4000 V capillary voltage, 500 V end plate offset, 600 Vpp funnel 1 RF, 400 Vpp funnel 2 RF, 80 V deflection delta, 5 eV ion energy, 10 eV collision energy, 1500 Vpp collision RF, 10 μs pre-pulse storage, 100 μs transfer time. TIMS (trapped ion mobility spectrometry) delta values were set to −20 V (delta 1), −120 V (delta 2), 80 V (delta 3), 100 V (delta 4), 0 V (delta 5), and 100 V (delta 6). The $1/k_0$ (inverse reduced ion mobility) range was set from 0.55 to 1.9 Vs/cm^2, the mass range was *m/z* 100–2000. Ion charge control (ICC) was enabled and set to 7.5 million counts. The samples were analyzed with TIMS ramp times of 100 ms. The analysis accumulation and ramp time was set at 100 ms with a spectra rate of 9.43 Hz and a total cycle of 0.32 s was also selected resulting in one full TIMS-MS scan. TIMS dimension was calibrated linearly using 4 selected ions from ESI Low Concentration Tuning Mix (Agilent Technologies) [*m/z*, $1/k_0$: (301.998139, 0.6678 Vs/cm^2), (601.979077, 0.8782 Vs/cm^2)] in negative mode and [*m/z*, $1/k_0$: (322.048121, 0.7363 Vs/cm^2), (622.028960, 0.9915 Vs/cm^2)] in positive mode. The mobility-mass correlation for calibration was taken from the CCS compendium (Picache et al., 2019).

4.6 | β-Galactosidase assay

For measurement of the activity of promoters fused to *lacZ*, an overnight culture of each strain was diluted 1:100 in 5 mL BHI broth and compounds were added where needed. Strains were grown at 25°C and 250 rpm until an OD_{600} of 0.6–0.8 and pelleted by centrifugation (11,000 × *g*, 2 min). The pellet was washed once in 600 μL ddH_2O and resuspended in 1200 μL PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate buffer, pH 7.4) buffer containing 0.15% β-mercaptoethanol. After sonification and centrifugation (11,000 × *g*, 5 min), 1000 μL of the supernatant was incubated at 30°C for 10 min. 200 μL 4 mg/mL ONPG in 1× PBS was added, and the reaction was incubated at 30°C for another 10 min, before being stopped by addition of 500 μL 1 M Na_2CO_3. Absorption was measured at 420 nm against PBS buffer incubated with ONPG as blank value. Protein concentration was determined by mixing 50 μL of the supernatant and 950 μL of a 1× Roti®-Nanoquant (Carl Roth, Karlsruhe, Germany) solution and measurement of the absorption at 595 nm against 50 μL 1× PBS as blank value. Afterward, the promoter activity in Miller units (MU) was calculated.

4.7 | Determination of minimal inhibitory concentrations

Minimal inhibitory concentrations (MIC) were determined in 96-well plates in a total volume of 200 μL. 200 μL BHI containing a two-fold dilution series of the antibiotic of interest were inoculated with overnight cultures at an initial OD_{600} of 0.05. The microtiter plates were incubated in a plate reader with intermittent shaking overnight at 37°C and growth was recorded for 20 h. The MIC was defined as the lowest concentration of the antibiotic at which no growth could be observed.

4.8 | Protein purification

Overexpression of *timR-strep* was performed in *E. coli* BL21. To this end, 500 mL LB broth containing 100 μg/mL ampicillin was inoculated with *E. coli* BL21 cells carrying the corresponding vector construct to an initial OD_{600} of 0.1. Cells were grown at 37°C and 250 rpm and 1 mM isopropyl-β-d-thiogalactopyranoside (IPTG) was added when an OD_{600} of 0.6–0.8 was reached. After three more hours, cells were pelleted by centrifugation (6000 × *g*, 5 min, 4°C) and the pellet was washed once with 25 mL buffer W (100 mM Tris–HCl pH 8.0; 150 mM NaCl). Cells were resuspended in 40 mL buffer W and lysed using an Emulsiflex homogenizer (Avestin, Germany). Cell debris was removed by centrifugation (6000 × *g*, 5 min, 4°C), and the resulting supernatant was filtered through a Minisart filter with a pore size of 0.45 μm (Sartorius). The strep-tagged protein was purified using affinity chromatography and Strep-Tactin Sepharose (IBA Lifesciences, Germany) according to the manufacturer's instructions. Fractions containing purified proteins were pooled, aliquoted, and stored at −20°C. Samples purity was analyzed by standard sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie staining.

4.9 | Electrophoretic mobility shift assay

To test the interaction of TimR with the P_{timABR} promoter, a 450 bp long sequence upstream of *timA* was synthesized by PCR from EGD-e genomic DNA using primers TE74 and TE75 and purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany). Likewise, a P_{divIVA} fragment was generated, but with SAH326 and TE425 as the primers. For the EMSA experiment, 32 nM of this purified promoter fragment was mixed with 5 µL of EMSA buffer (120 mM HEPES, 300 mM KCl, 30 mM $MgCl_2$, 0.3 mg/mL bovine serum albumin [BSA], 30% glycerol, 0.3 mM EDTA, pH 8.0) and incubated with varying amounts of TimR-Strep, tartrolon A, tartrolon B and boromycin for 5 min at room temperature. The reaction was loaded onto an acrylamide gel (10% (w/v) acrylamide with 0.6× Tris-borate EDTA) and run at 120 V for 75 min. Afterward, the gel was stained for 5 min using ethidium bromide and photographed using an UV transilluminator.

AUTHOR CONTRIBUTIONS

Sven Halbedel: Conceptualization; formal analysis; supervision; funding acquisition; project administration; writing – original draft; visualization. **Tim Engelgeh:** Investigation; data curation; validation; formal analysis; writing – original draft. **Jennifer Herrmann:** Methodology; data curation; writing – original draft; validation; project administration. **Rolf Jansen:** Methodology; investigation; validation; formal analysis; writing – original draft. **Rolf Müller:** Methodology; project administration; writing – original draft; supervision.

ACKNOWLEDGEMENTS

This work was supported by a grant of the DFG (HA6830/2-1) to S. H. The authors are grateful to all group members for fruitful discussions. We would also like to thank Kerstin Schober and Viktoria George for excellent technical assistance with natural products supply and Sabine Backes, Dr. Jake Haeckl and Dr. Susanne Kirsch-Dahmen for support of analytics. Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This article does not include research on humans or animals.

ORCID

Sven Halbedel https://orcid.org/0000-0002-5575-8973

REFERENCES

Aase, B., Sundheim, G., Langsrud, S. & Rorvik, L.M. (2000) Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in *Listeria monocytogenes*. *International Journal of Food Microbiology*, 62, 57–63. Available from: https://doi.org/10.1016/s0168-1605(00)00357-3

Arcari, T., Feger, M.L., Guerreiro, D.N., Wu, J. & O'Byrne, C.P. (2020) Comparative review of the responses of *Listeria monocytogenes* and *Escherichia coli* to low pH stress. *Genes (Basel)*, 11, 1330. Available from: https://doi.org/10.3390/genes11111330

Arnaud, M., Chastanet, A. & Debarbouille, M. (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Applied and Environmental Microbiology*, 70, 6887–6891. Available from: https://doi.org/10.1128/AEM.70.11.6887-6891.2004

Bertram, R., Neumann, B. & Schuster, C.F. (2021) Status quo of tet regulation in bacteria. *Microbial Biotechnology*, 15, 1101–1119. Available from: https://doi.org/10.1111/1751-7915.13926

Bou-m'handi, N., Jacquet, C., El Marrakchi, A. & Martin, P. (2007) Phenotypic and molecular characterization of *Listeria monocytogenes* strains isolated from a marine environment in Morocco. *Foodborne Pathogens and Disease*, 4, 409–417. Available from: https://doi.org/10.1089/fpd.2007.0019

Bucur, F.I., Grigore-Gurgu, L., Crauwels, P., Riedel, C.U. & Nicolau, A.I. (2018) Resistance of *Listeria monocytogenes* to stress conditions encountered in food and food processing environments. *Frontiers in Microbiology*, 9, 2700. Available from: https://doi.org/10.3389/fmicb.2018.02700

Cavanagh, D., Fitzgerald, G.F. & McAuliffe, O. (2015) From field to fermentation: the origins of *Lactococcus lactis* and its domestication to the dairy environment. *Food Microbiology*, 47, 45–61. Available from: https://doi.org/10.1016/j.fm.2014.11.001

Charlier, C., Perrodeau, E., Leclercq, A., Cazenave, B., Pilmis, B., Henry, B. et al. (2017) Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *The Lancet Infectious Diseases*, 17, 510–519. Available from: https://doi.org/10.1016/S1473-3099(16)30521-7

Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B.L. et al. (2002) Structural identification of a bacterial quorum-sensing signal containing boron. *Nature*, 415, 545–549. Available from: https://doi.org/10.1038/415545a

Collins, B., Curtis, N., Cotter, P.D., Hill, C. & Ross, R.P. (2010) The ABC transporter AnrAB contributes to the innate resistance of *Listeria monocytogenes* to nisin, bacitracin, and various beta-lactam antibiotics. *Antimicrobial Agents and Chemotherapy*, 54, 4416–4423. Available from: https://doi.org/10.1128/AAC.00503-10

Crimmins, G.T., Herskovits, A.A., Rehder, K., Sivick, K.E., Lauer, P., Dubensky, T.W., Jr. et al. (2008) *Listeria monocytogenes* multidrug resistance transporters activate a cytosolic surveillance pathway of innate immunity. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 10191–10196. Available from: https://doi.org/10.1073/pnas.0804170105

den Bakker, H.C., Warchocki, S., Wright, E.M., Allred, A.F., Ahlstrom, C., Manuel, C.S. et al. (2014) *Listeria floridensis* sp. nov., *Listeria aquatica* sp. nov., *Listeria cornellensis* sp. nov., *Listeria riparia* sp. nov. and *Listeria grandensis* sp. nov., from agricultural and natural environments. *International Journal of Systematic and Evolutionary Microbiology*, 64, 1882–1889. Available from: https://doi.org/10.1099/ijs.0.052720-0

Dillon, R.M. & Patel, T.R. (1992) Listeria in Seafoods: a review. *Journal of Food Protection*, 55, 1009–1015. Available from: https://doi.org/10.4315/0362-028X-55.12.1009

DZIF/TTU9. (2023) Compound resources and medicinal chemistry. https://www.dzif.de/en/compound-resources-and-medicinal-chemistry

Elshahawi, S.I., Trindade-Silva, A.E., Hanora, A., Han, A.W., Flores, M.S., Vizzoni, V. et al. (2013) Boronated tartrolon antibiotic produced by symbiotic cellulose-degrading bacteria in shipworm gills. *Proceedings of the National Academy of Sciences of the United States of America*, 110, E295–E304. Available from: https://doi.org/10.1073/pnas.1213892110

El-Shenawy, M.A. & El-Shenawy, M.A. (2006) *Listeria spp*. in the coastal environment of the Aqaba gulf, Suez gulf and the Red Sea. *Epidemiology and Infection*, 134, 752–757. Available from: https://doi.org/10.1017/S0950268805005601

Epstein, W. (2003) The roles and regulation of potassium in bacteria. *Progress in Nucleic Acid Research and Molecular Biology*, 75, 293–320. Available from: https://doi.org/10.1016/s0079-6603(03)75008-9

Esteban, J.I., Oporto, B., Aduriz, G., Juste, R.A. & Hurtado, A. (2009) Faecal shedding and strain diversity of *Listeria monocytogenes* in healthy ruminants and swine in Northern Spain. *BMC Veterinary Research*, 5, 2. Available from: https://doi.org/10.1186/1746-6148-5-2

Freitag, N.E., Port, G.C. & Miner, M.D. (2009) *Listeria monocytogenes*—from saprophyte to intracellular pathogen. *Nature Revirws Microbiology*, 7, 623–628. Available from: https://doi.org/10.1038/nrmicro2171

Gahan, C.G. & Hill, C. (2014) *Listeria monocytogenes*: survival and adaptation in the gastrointestinal tract. *Frontiers in Cellular and Infection Microbiology*, 4, 9. Available from: https://doi.org/10.3389/fcimb.2014.00009

Gilchrist, C.L.M., Booth, T.J., van Wersch, B., van Grieken, L., Medema, M.H. & Chooi, Y.H. (2021) Cblaster: a remote search tool for rapid identification and visualization of homologous gene clusters. *Bioinformatics Advances*, 1, vbab016. Available from: https://doi.org/10.1093/bioadv/vbab016

Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F. et al. (2001) Comparative genomics of *Listeria* species. *Science*, 294, 849–852. Available from: https://doi.org/10.1126/science.1063447294/5543/849

Godreuil, S., Galimand, M., Gerbaud, G., Jacquet, C. & Courvalin, P. (2003) Efflux pump Lde is associated with fluoroquinolone resistance in *Listeria monocytogenes*. *Antimicrobial Agents and Chemotherapy*, 47, 704–708. Available from: 10.1128/AAC.47.2.704-708.2003

Gräfe, U., Schlegel, R., Ritzau, M., Ihn, W., Dornberger, K., Stengel, C. et al. (1995) Aurantimycins, new depsipeptide antibiotics from *Streptomyces aurantiacus* IMET 43917. Production, isolation, structure elucidation, and biological activity. *Journal of Antibiotics (Tokyo)*, 48, 119–125.

Grigoriev, P., Schlegel, R., Dornberger, K. & Gräfe, U. (1995) Formation of membrane pores by aurantimycins A and B, new lipopeptide antibiotics from *Streptomyces aurantiacus*. *Bioelectrochemistry and Bioenergetics*, 36, 57–59. Available from: https://doi.org/10.1016/0302-4598(94)01721-C

Grubaugh, D., Regeimbal, J.M., Ghosh, P., Zhou, Y., Lauer, P., Dubensky, T.W., Jr. et al. (2018) The VirAB ABC transporter is required for VirR regulation of *Listeria monocytogenes* virulence and resistance to Nisin. *Infection and Immunity*, 86, e00901-17. Available from: https://doi.org/10.1128/IAI.00901-17

Guerin, F., Galimand, M., Tuambilangana, F., Courvalin, P. & Cattoir, V. (2014) Overexpression of the novel MATE fluoroquinolone efflux pump FepA in *listeria monocytogenes* is driven by inactivation of its local repressor FepR. *PLoS ONE*, 9, e106340. Available from: https://doi.org/10.1371/journal.pone.0106340

Gundlach, J., Herzberg, C., Hertel, D., Thurmer, A., Daniel, R., Link, H. et al. (2017) Adaptation of Bacillus subtilis to life at extreme potassium limitation. *mBio*, 8, e00861-17. Available from: 10.1128/mBio.00861-17

Gupta, A., Pande, A., Sabrin, A., Thapa, S.S., Gioe, B.W. & Grove, A. (2018) MarR family transcription factors from *Burkholderia* species: hidden clues to control of virulence-associated genes. *Microbiology and Molecular Biology Reviews*, 83, e00039-18. Available from: https://doi.org/10.1128/MMBR.00039-18

Hafner, L., Pichon, M., Burucoa, C., Nusser, S.H.A., Moura, A., Garcia-Garcera, M. et al. (2021) *Listeria monocytogenes* faecal carriage is common and depends on the gut microbiota. *Nature Communications*, 12, 6826. Available from: https://doi.org/10.1038/s41467-021-27069-y

Hamoen, L.W., Smits, W.K., de Jong, A., Holsappel, S. & Kuipers, O.P. (2002) Improving the predictive value of the competence transcription factor (ComK) binding site in *Bacillus subtilis* using a genomic approach. *Nucleic Acids Research*, 30, 5517–5528.

Hauf, S., Engelgeh, T. & Halbedel, S. (2021) Elements in the LftR repressor operator Interface contributing to regulation of Aurantimycin resistance in *Listeria monocytogenes*. *Journal of Bacteriology*, 203, e00503-20. Available from: https://doi.org/10.1128/JB.00503-20

Hauf, S., Herrmann, J., Miethke, M., Gibhardt, J., Commichau, F.M., Müller, R. et al. (2019) Aurantimycin resistance genes contribute to survival of *listeria monocytogenes* during life in the environment. *Molecular Microbiology*, 111, 1009–1024. Available from: https://doi.org/10.1111/mmi.14205

Housseini, B.I.K., Phan, G. & Broutin, I. (2018) Functional mechanism of the efflux pumps transcription regulators from *Pseudomonas aeruginosa* based on 3D structures. *Frontiers in Molecular Biosciences*, 5, 57. Available from: https://doi.org/10.3389/fmolb.2018.00057

Hütter, R., Keller-Schien, W., Knüsel, F., Prelog, V., Rodgers jr., G.C., Suter, P. et al. (1967) Stoffwechselprodukte von Mikroorganismen. 57. Mitteilung. Boromycin. *Helvetica Chimica Acta*, 50, 1533–1539. Available from: https://doi.org/10.1002/hlca.19670500612

Irschik, H., Schummer, D., Gerth, K., Hofle, G. & Reichenbach, H. (1995) The tartrolons, new boron-containing antibiotics from a myxobacterium, *Sorangium cellulosum*. *Journal of Antibiotics (Tokyo)*, 48, 26–30. Available from: https://doi.org/10.7164/antibiotics.48.26

Jain, D. (2015) Allosteric control of transcription in GntR family of transcription regulators: a structural overview. *IUBMB Life*, 67, 556–563. Available from: https://doi.org/10.1002/iub.1401

Jiang, X., Geng, Y., Ren, S., Yu, T., Li, Y., Liu, G. et al. (2019) The VirAB-VirSR-AnrAB multicomponent system is involved in resistance of *Listeria monocytogenes* EGD-e to cephalosporins, bacitracin, nisin, benzalkonium chloride, and ethidium bromide. *Applied and Environmental Microbiology*, 85, e01470-19. Available from: https://doi.org/10.1128/AEM.01470-19

Kaplan Zeevi, M., Shafir, N.S., Shaham, S., Friedman, S., Sigal, N., Nir Paz, R. et al. (2013) Listeria monocytogenes multidrug resistance transporters and cyclic di-AMP, which contribute to type I interferon induction, play a role in cell wall stress. *Journal of Bacteriology*, 195, 5250–5261. Available from: https://doi.org/10.1128/JB.00794-13

Kaur, G., Malik, R.K., Mishra, S.K., Singh, T.P., Bhardwaj, A., Singroha, G. et al. (2011) Nisin and class IIa bacteriocin resistance among *Listeria* and other foodborne pathogens and spoilage bacteria. *Microbial Drug Resistance*, 17, 197–205. Available from: https://doi.org/10.1089/mdr.2010.0054

Kaval, K.G., Hahn, B., Tusamda, N., Albrecht, D. & Halbedel, S. (2015) The PadR-like transcriptional regulator LftR ensures efficient invasion of *Listeria monocytogenes* into human host cells. *Frontiers in Microbiology*, 6, 772. Available from: https://doi.org/10.3389/fmicb.2015.00772

Kellner, R., Jung, G., Horner, T., Zahner, H., Schnell, N., Entian, K.D. et al. (1988) Gallidermin: a new lanthionine-containing polypeptide antibiotic. *European Journal of Biochemistry*, 177, 53–59. Available from: https://doi.org/10.1111/j.1432-1033.1988.tb14344.x

Kopp, F. & Marahiel, M.A. (2007) Macrocyclization strategies in polyketide and nonribosomal peptide biosynthesis. *Natural Product Reports*, 24, 735–749. Available from: https://doi.org/10.1039/b613652b

Kuroda, T. & Tsuchiya, T. (2009) Multidrug efflux transporters in the MATE family. *Biochimica et Biophysica Acta*, 1794, 763–768. Available from: https://doi.org/10.1016/j.bbapap.2008.11.012

Lachmann, R., Halbedel, S., Lüth, S., Holzer, A., Adler, M., Pietzka, A. et al. (2022) Invasive listeriosis outbreaks and salmon products: a genomic, epidemiological study. *Emerging Microbes and Infections*,

11, 1308–1315. Available from: https://doi.org/10.1080/22221751.2022.2063075

Lakicevic, B.Z., Den Besten, H.M.W. & De Biase, D. (2021) Landscape of stress response and virulence genes among *Listeria monocytogenes* strains. *Frontiers in Microbiology*, 12, 738470. Available from: https://doi.org/10.3389/fmicb.2021.738470

Lewis, P.J. & Marston, A.L. (1999) GFP vectors for controlled expression and dual labelling of protein fusions in *Bacillus subtilis*. *Gene*, 227, 101–110.

Lubelski, J., Konings, W.N. & Driessen, A.J. (2007) Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiology and Molecular Biology Reviews*, 71, 463–476. Available from: https://doi.org/10.1128/MMBR.00001-07

Lubelski, J., Rink, R., Khusainov, R., Moll, G.N. & Kuipers, O.P. (2008) Biosynthesis, immunity, regulation, mode of action and engineering of the model lantibiotic nisin. *Cellular and Molecular Life Sciences*, 65, 455–476. Available from: https://doi.org/10.1007/s00018-007-7171-2

Mata, M.T., Baquero, F. & Perez-Diaz, J.C. (2000) A multidrug efflux transporter in *Listeria monocytogenes*. *FEMS Microbiology Letters*, 187, 185–188.

Monk, I.R., Gahan, C.G. & Hill, C. (2008) Tools for functional postgenomic analysis of *Listeria monocytogenes*. *Applied and Environmental Microbiology*, 74, 3921–3934. Available from: https://doi.org/10.1128/AEM.00314-08

Moreira, W., Aziz, D.B. & Dick, T. (2016) Boromycin kills mycobacterial persisters without detectable resistance. *Frontiers in Microbiology*, 7, 199. Available from: https://doi.org/10.3389/fmicb.2016.00199

Müller, A., Rychli, K., Zaiser, A., Wieser, C., Wagner, M. & Schmitz-Esser, S. (2014) The *Listeria monocytogenes* transposon Tn6188 provides increased tolerance to various quaternary ammonium compounds and ethidium bromide. *FEMS Microbiology Letters*, 361, 166–173. Available from: https://doi.org/10.1111/1574-6968.12626

Newman, H., Krajnc, A., Bellini, D., Eyermann, C.J., Boyle, G.A., Paterson, N.G. et al. (2021) High-throughput crystallography reveals boron-containing inhibitors of a penicillin-binding protein with Di- and Tricovalent binding modes. *Journal of Medicinal Chemistry*, 64, 11379–11394. Available from: https://doi.org/10.1021/acs.jmedchem.1c00717

Nguyen, L., Schultz, D.C., Terzyan, S.S., Rezaei, M., Songb, J., Li, C. et al. (2022) Design and evaluation of novel analogs of 2-amino-4-boronobutanoic acid (ABBA) as inhibitors of human gamma-glutamyl transpeptidase. *Bioorganic & Medicinal Chemistry*, 73, 116986. Available from: https://doi.org/10.1016/j.bmc.2022.116986

Nolan, D.A., Chamblin, D.C. & Troller, J.A. (1992) Minimal water activity levels for growth and survival of *Listeria monocytogenes* and *Listeria innocua*. *International Journal of Food Microbiology*, 16, 323–335. Available from: https://doi.org/10.1016/0168-1605(92)90034-z

Orsi, R.H. & Wiedmann, M. (2016) Characteristics and distribution of Listeria spp., including *Listeria* species newly described since 2009. *Applied Microbiology and Biotechnology*, 100, 5273–5287. Available from: https://doi.org/10.1007/s00253-016-7552-2

Pache, W. & Zähner, H. (1969) Metabolic products of microorganisms. 77. Studies on the mechanism of action of boromycin. *Archiv für Mikrobiologie*, 67, 156–165.

Perez, M., Crespo, C., Schleissner, C., Rodriguez, P., Zuniga, P. & Reyes, F. (2009) Tartrolon D, a cytotoxic macrodiolide from the marine-derived actinomycete *Streptomyces sp*. MDG-04-17-069. *Journal of Natural Products*, 72, 2192–2194. Available from: https://doi.org/10.1021/np9006603

Picache, J.A., Rose, B.S., Balinski, A., Leaptrot, K.L., Sherrod, S.D., May, J.C. et al. (2019) Collision cross section compendium to annotate and predict multi-omic compound identities. *Chemical Science*, 10, 983–993. Available from: https://doi.org/10.1039/c8sc04396e

Quereda, J.J., Moron-Garcia, A., Palacios-Gorba, C., Dessaux, C., Garcia-Del Portillo, F., Pucciarelli, M.G. et al. (2021) Pathogenicity and virulence of *Listeria monocytogenes*: a trip from environmental to medical microbiology. *Virulence*, 12, 2509–2545. Available from: https://doi.org/10.1080/21505594.2021.1975526

Quereda, J.J., Nahori, M.A., Meza-Torres, J., Sachse, M., Titos-Jimenez, P., Gomez-Laguna, J. et al. (2017) Listeriolysin S is a streptolysin S-like virulence factor that targets exclusively prokaryotic cells *In vivo*. *mBio*, 8, e00259-17. Available from: https://doi.org/10.1128/mBio.00259-17

Ren, Q., Chen, K. & Paulsen, I.T. (2007) TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. *Nucleic Acids Research*, 35, D274–D279. Available from: https://doi.org/10.1093/nar/gkl925

Ren, Q., Kang, K.H. & Paulsen, I.T. (2004) TransportDB: a relational database of cellular membrane transport systems. *Nucleic Acids Research*, 32, D284–D288. Available from: https://doi.org/10.1093/nar/gkh016

Rodas-Suarez, O.R., Flores-Pedroche, J.F., Betancourt-Rule, J.M., Quinones-Ramirez, E.I. & Vazquez-Salinas, C. (2006) Occurrence and antibiotic sensitivity of *Listeria monocytogenes* strains isolated from oysters, fish, and estuarine water. *Applied and Environmental Microbiology*, 72, 7410–7412. Available from: https://doi.org/10.1128/AEM.00956-06

Rolhion, N., Chassaing, B., Nahori, M.A., de Bodt, J., Moura, A., Lecuit, M. et al. (2019) A *Listeria monocytogenes* Bacteriocin can target the commensal prevotella copri and modulate intestinal infection. *Cell Host & Microbe*, 26, 691–701 e695. Available from: https://doi.org/10.1016/j.chom.2019.10.016

Ruppitsch, W., Pietzka, A., Prior, K., Bletz, S., Fernandez, H.L., Allerberger, F. et al. (2015) Defining and evaluating a core genome multilocus sequence typing scheme for whole-genome sequence-based typing of *Listeria monocytogenes*. *Journal of Clinical Microbiology*, 53, 2869–2876. Available from: https://doi.org/10.1128/JCM.01193-15

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular cloning: a laboratory manual*. Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press, p. 3.

Saraylou, M., Nadian Ghomsheh, H., Enayatizamir, N., Rangzan, N. & St. Clair Senn, S. (2021) Some plant growth promoting traits of Streptomyces species isolated from various crop rhizospheres with high root colonization ability of spinach (*Spinacia oleracea* L.). *Applied Ecology and Environmental Research*, 19, 3069–3081. Available from: https://doi.org/10.15666/aeer/1904_30693081

Scheldeman, P., Herman, L., Foster, S. & Heyndrickx, M. (2006) *Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk. *Journal of Applied Microbiology*, 101, 542–555. Available from: https://doi.org/10.1111/j.1365-2672.2006.02964.x

Schoder, D., Guldimann, C. & Martlbauer, E. (2022) Asymptomatic carriage of *Listeria monocytogenes* by animals and humans and its impact on the food chain. *Food*, 11, 3472. Available from: https://doi.org/10.3390/foods11213472

Schummer, D., Irschik, H., Reichenbach, H. & Höfle, G. (1994) Antibiotics from gliding bacteria, LVII. Tartrolons: new boron-containing macrodiolides from *Sorangium cellulosum*. *Liebigs Annalen der Chemie*, 1994, 283–289. Available from: https://doi.org/10.1002/jlac.199419940310

Shahamat, M., Seaman, A. & Woodbine, M. (1980) Survival of *listeria monocytogenes* in high salt concentrations. *Zentralbl Bakteriol A*, 246, 506–511.

Sleator, R.D., Wemekamp-Kamphuis, H.H., Gahan, C.G., Abee, T. & Hill, C. (2005) A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in *Listeria monocytogenes*. *Molecular Microbiology*,

55, 1183–1195. Available from: https://doi.org/10.1111/j.1365-2958.2004.04454.x

Solovyev, V. & Salamov, A. (2011) Automatic annotation of microbial genomes and metagenomic sequences. In: Li, R.W. (Ed.) *Metagenomics and its applications in agriculture, biomedicine and environmental studies*. Hauppauge, N.Y: Nova Science Publishers, pp. 61–78.

Tiensuu, T., Guerreiro, D.N., Oliveira, A.H., O'Byrne, C. & Johansson, J. (2019) Flick of a switch: regulatory mechanisms allowing *Listeria monocytogenes* to transition from a saprophyte to a killer. *Microbiology*, 165, 819–833. Available from: https://doi.org/10.1099/mic.0.000808

Toledo-Arana, A., Dussurget, O., Nikitas, G., Sesto, N., Guet-Revillet, H., Balestrino, D. et al. (2009) The *listeria* transcriptional landscape from saprophytism to virulence. *Nature*, 459, 950–956. Available from: https://doi.org/10.1038/nature08080

Truong, H.N., Garmyn, D., Gal, L., Fournier, C., Sevellec, Y., Jeandroz, S. et al. (2021) Plants as a realized niche for listeria monocytogenes. *MicrobiologyOpen*, 10, e1255. Available from: https://doi.org/10.1002/mbo3.1255

van den Ent, F. & Löwe, J. (2006) RF cloning: a restriction-free method for inserting target genes into plasmids. *Journal of Biochemical and Biophysical Methods*, 67, 67–74. Available from: https://doi.org/10.1016/j.jbbm.2005.12.008

Vazquez-Boland, J.A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W. et al. (2001) *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews*, 14, 584–640. Available from: https://doi.org/10.1128/CMR.14.3.584-640.2001

Vijayabharathi, R., Bruheim, P., Andreassen, T., Raja, D.S., Devi, P.B., Sathyabama, S. et al. (2011) Assessment of resistomycin, as an anticancer compound isolated and characterized from *Streptomyces aurantiacus* AAA5. *Journal of Microbiology*, 49, 920–926. Available from: https://doi.org/10.1007/s12275-011-1260-5

Vivant, A.L., Garmyn, D. & Piveteau, P. (2013) *Listeria monocytogenes*, a down-to-earth pathogen. *Frontiers in Cellular and Infection Microbiology*, 3, 87. Available from: https://doi.org/10.3389/fcimb.2013.00087

Wendlandt, S., Shen, J., Kadlec, K., Wang, Y., Li, B., Zhang, W.J. et al. (2015) Multidrug resistance genes in staphylococci from animals that confer resistance to critically and highly important antimicrobial agents in human medicine. *Trends in Microbiology*, 23, 44–54. Available from: https://doi.org/10.1016/j.tim.2014.10.002

Wilking, H., Lachmann, R., Holzer, A., Halbedel, S., Flieger, A. & Stark, K. (2021) Ongoing high incidence and case-fatality rates for invasive Listeriosis, Germany, 2010–2019. *Emerging Infectious Diseases*, 27, 2485–2488. Available from: https://doi.org/10.3201/eid2709.210068

Woodward, J.J., Iavarone, A.T. & Portnoy, D.A. (2010) c-di-AMP secreted by intracellular *Listeria monocytogenes* activates a host type I interferon response. *Science*, 328, 1703–1705. Available from: https://doi.org/10.1126/science.1189801

Wurtzel, O., Sesto, N., Mellin, J.R., Karunker, I., Edelheit, S., Becavin, C. et al. (2012) Comparative transcriptomics of pathogenic and non-pathogenic listeria species. *Molecular Systems Biology*, 8, 583. Available from: https://doi.org/10.1038/msb.2012.11

Yin, X. & Zabriskie, T.M. (2006) The enduracidin biosynthetic gene cluster from *Streptomyces fungicidicus*. *Microbiology (Reading)*, 152, 2969–2983. Available from: https://doi.org/10.1099/mic.0.29043-0

Young, J. & Holland, I.B. (1999) ABC transporters: bacterial exporters-revisited five years on. *Biochimica et Biophysica Acta*, 1461, 177–200. Available from: https://doi.org/10.1016/s0005-2736(99)00158-3

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Engelgeh, T., Herrmann, J., Jansen, R., Müller, R. & Halbedel, S. (2023) Tartrolon sensing and detoxification by the *Listeria monocytogenes timABR* resistance operon. *Molecular Microbiology*, *120*, 629–644. Available from: https://doi.org/10.1111/mmi.15178