

Analysis of two pheromone-responsive conjugative multiresistance plasmids carrying the novel mobile *optrA* locus from *Enterococcus faecalis*

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Background: The acquired *optrA* gene, which encodes a ribosomal protection protein of the ABC-F family, can confer cross-resistance to linezolid and florfenicol, posing a serious therapeutic challenge to both human and veterinary medicine.

Purpose: The objective of this study was to investigate the two *Enterococcus faecalis* (*E. faecalis*) plasmids for their fine structure, their transferability and the presence of mobile antimicrobial resistance loci.

Methods: To elucidate their fine structure, the two plasmids were completely sequenced and the sequences analysed. Besides conjugation experiments, inverse PCR assays were conducted to see whether minicircles are produced from the mobile antimicrobial resistance loci.

Results: Two pheromone-responsive conjugative *optrA*-carrying plasmids from *E. faecalis*, pE211 and pE508 were identified, which can transfer with frequencies of 2.6×10^{-2} and 3.7×10^{-2} (transconjugant per donor), respectively. In both plasmids, *optrA* was located on the novel mobile *optrA* locus with different sizes (12,834 bp in pE211 and 7,561 bp in pE508, respectively), flanked by two copies of *IS1216* genes in the same orientation. Inverse PCR revealed that circular forms can be generated, consisting of *optrA* and one copy of *IS1216*, indicating they are all active. The 77,562 bp plasmid pE211 also carried *Tn558* and a mobile *bcxABDR* locus, and the 84,468 bp plasmid pE508 also harbored the genes *fexA*, *tet(L)*, *tet* (O/W/32/O) and a mobile *aac(A)-aph(D)* locus.

Conclusion: The presence of mobile genetic elements in these plasmids renders them flexible and these elements will aid to the persistence and dissemination of these plasmids among enterococci and potentially also other gram-positive bacteria.

Keywords: enterococci, resistance, *IS1216*, conjugation, mobile genetic elements

Introduction

Linezolid and florfenicol are both important antimicrobial agents. Linezolid is approved in human medicine and usually used as a last resort antimicrobial agent to treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).¹ Florfenicol is approved exclusively for food-producing animals, where it is mainly used for the control of respiratory tract infections.² However, the acquired cross-resistance to linezolid and florfenicol has emerged during the past decade.^{3–5}

Currently, at least three different groups of acquired resistance genes which confer cross-resistance to linezolid and florfenicol have been identified. These include *cfi*, *optrA* and *poxtA*.^{3–5} Among them, the *optrA* gene encodes a ribosomal protection protein of the

ABC-F family.⁶ It was first found in *Enterococcus faecalis* and *Enterococcus faecium*,⁴ but has also been detected meanwhile in various Gram-positive bacteria.^{7,8}

In this study, two pheromone-responsive conjugative plasmids harboring *optrA* along with other resistance genes were analyzed to elucidate the basis for co-selection and dissemination. Furthermore, two novel mobile *optrA* loci in these plasmids were identified.

Materials and methods

Bacterial strains and antimicrobial susceptibility testing

Two *optrA*-positive *E. faecalis* strains (E211–ST59 and E508–ST256) were identified from fecal samples of swine in Henan Province, China during a routine survey in 2015. Antimicrobial susceptibility testing was performed by broth microdilution according to the recommendations given in document M100 (28th ed.) of the Clinical and Laboratory Standards Institute (CLSI).⁹ *S. aureus* ATCC 29213 served as the quality control strain.

PCR analysis

The presence of the *optrA* gene was detected by PCR using the primers listed in Table 1. The *optrA*-carrying plasmid pE349 was used as the positive control.⁴ The presence of the circular intermediate was detected by inverse PCR using the primers listed in Table 1. All the PCR products were subjected to Sanger sequencing.

Transfer experiments

To investigate the transferability of the *optrA* gene, these two *E. faecalis* strains were used in conjugation experiments with

E. faecalis JH2-2 (rifampicin resistant) as the recipient.¹⁰ Transfer frequency is expressed as transconjugant per donor. Colonies that grew on the selective plates supplemented with 50 mg/L rifampin and 10 mg/L florfenicol after incubation for 16–24 h at 37°C were further confirmed by antimicrobial susceptibility testing and multilocus sequence typing (MLST) following harmonized protocols (<http://www.mlst.net/>).

Sequencing and analysis

Whole genome DNA of two *optrA*-positive transconjugants E211-T1 and E508-T1 was sequenced by the PacBio RS and Illumina MiSeq platforms. The sequences from PacBio sequencing reads were *de-novo* assembled and corrected by Illumina MiSeq with pilon. Glimmer 3.02 was used to predict open reading frames (ORFs) and the software blast was used to annotate those ORFs. The sequences determined had been deposited in GenBank under accession numbers MK425644 (pE211) and MK425645 (pE508), respectively.

Results and discussion

The *optrA* gene in *E. faecalis* is transferable

The conjugation experiments indicated that these two *E. faecalis* strains (E211–ST59 and E508–ST256) could transfer florfenicol resistance to the recipient *E. faecalis* JH2-2 (ST8) at high transfer frequencies, of 2.6×10^{-2} for *E. faecalis* E211 and 3.7×10^{-2} for *E. faecalis* E508 (transconjugant per donor), respectively. Two transconjugants which were confirmed to share the same background with the recipient (ST8), designated E211-T1 and E508-T1, respectively, were selected for further

Table 1 PCR primers used in this study

Category and gene	Primer designation	Sequence (5'-3')	Product size (bp)	Reference or source
<i>optrA</i>	<i>optrA</i> -fw <i>optrA</i> -rv	GCACCAGACCAATACGATACAA TCCTTCTTAACCTTCTCCTTCTCA	794	This study
<i>optrA</i> minicircle in plasmid pE211	circ-I-fw circ-I-rv	TATCAAGCGAAATATGCAGG TGCACCATTTAGCTTTTCGT	4,052	This study
<i>bcrABDR</i> minicircle	circ-II-fw circ-II-rv	AAATGGGTATGGGCAATATG ATCGCTTGTGGCTATATCA	4,633	This study
Tn558 minicircle	circ-III-fw circ-III-rv	CGGTGCCTAATCATTCTGATGC CGCTTAACCGTTCTATCACTTCA		11
<i>optrA</i> minicircle in plasmid pE508	circ-IV-fw circ-IV-rv	TGCACATACTTGAAACCTCC CTTGAACACTGATTCTCGG	3,601	This study
<i>aac(A)-aph(D)</i> minicircle	circ-V-fw circ-V-rv	TGCCACACTATCATAACCACT ACTTTTAATTCTAGCGTGCCT	3,227	This study

Table 2 The antimicrobial susceptibilities of the wild-type strains, transconjugants, and recipient strains in this study

Strains	MICs(mg/L) ^a				
	FFC	LZD	BAC	GEN	TET
E211	128	8	128	>128	128
E211-T1 ^b	128	8	128	8	1
E508	128	8	4	>128	128
E508-T1 ^b	128	8	2	>128	64
JH2-2	4	2	2	8	1

Notes: ^aFFC, florfenicol; LZD, linezolid; TET, tetracycline; GEN, gentamicin; BAC, bacitracin. ^bThe transconjugants E211-T1 and E508-T1 were derived from matings between *E. faecalis* strains E211/E508 and JH2-2, respectively.

studies. Sequencing and sequence analysis identified two conjugative *optrA*-carrying plasmids, designated pE211 and pE508, which were derived from E211-T1 and E508-T1, respectively. The conjugative transfer region in both plasmids pE211 and pE508 displayed the greatest similarity with that in plasmid pTW9, which has key conjugative properties of pheromone-responsive plasmids, such as aggregation substance (As). In combination with their high transfer frequencies, these two plasmids (pE211 and pE508) can be classified as pheromone-responsive conjugative plasmids. MICs of the two *E. faecalis* strains, their transconjugants and the recipient strain are shown in Table 2. After transfer, the transconjugants displayed elevated MICs to the respective antimicrobial agents, including florfenicol, linezolid and bacitracin in E211-T1, and florfenicol, linezolid, gentamicin and tetracycline in E508-T1. As shown in Table 3, although there are few amino acid substitutions, the linezolid resistance that the *OptrA* variants in *E. faecalis* E211 or E508 confer remains the same with the *OptrA* prototype in *E. faecalis* E349.⁴

Both plasmids pE211 and pE508 have a novel mobile *optrA* locus

The IS1216-flanked *optrA* locus in plasmid pE211 consisted of the transcriptional regulator gene *araC*, the *optrA* gene and

Table 3 Comparison the difference of *OptrA* variants and MICs with wide-type strain

Strain	<i>OptrA</i> variant	Amino acid substitutions	MICs of linezolid (mg/L)	References
E349	Wide-type	none	8	4
E211	ED	K3E, G393D	8	This study
E508	DP	Y176D, T481P	8	This study

a restriction endonuclease gene (MGE1 in Figure 1A, 12,834 bp), while that in plasmid pE508 carried the *optrA* gene and a truncated *erm(A)*-like gene (MGE3 in Figure 1B, 7,561 bp). In both plasmids, *optrA* was flanked by two copies of IS1216 genes located in the same orientation, forming a novel locus, which was different from that described in previous studies (Figure 2).^{11,12} In both cases, the two IS1216 elements can recombine and “loop out” a circular intermediate, which can then integrate either into plasmids or in the chromosomal DNA by recombination with another IS1216 copy. Via this way, the *optrA* gene can move between different chromosomal and plasmidic locations. If finally integrated into a conjugative plasmid or ICE, it can move with this element across strain, species or even genus boundaries. To investigate whether circular intermediates were present, inverse PCR assays using the primers listed in Table 1 were conducted and the results showed that circular intermediates of different sizes (4,052 bp in plasmid pE211 and 3,601 bp in plasmid pE508) were formed in these strains. Sequence analysis of these circular intermediates confirmed that they contained one copy of the IS1216 element and the sequence that was formerly located between the two IS1216 elements, including *optrA*.

The IS1216-like elements have also been reported to be associated with the vancomycin resistance *VanA* gene cluster in *E. faecium*,¹³ the multidrug resistance genes *poxtA*, *optrA* and *cfp* in enterococci and staphylococci,^{5,11,14} the macrolide-lincosamide-streptogramin B resistance genes *erm(B)* and *erm(T)* in enterococci and streptococci,^{15,16} and the tetracycline resistance gene *tet(S)* in *Streptococcus infantis*.¹⁷ These observations, along with multiple MGEs (MGE1-MGE4) associating with IS1216 elements found in this study, suggest that the IS1216-like elements could play an important role in dissemination of the respective antimicrobial resistance genes among various Gram-positive organisms.

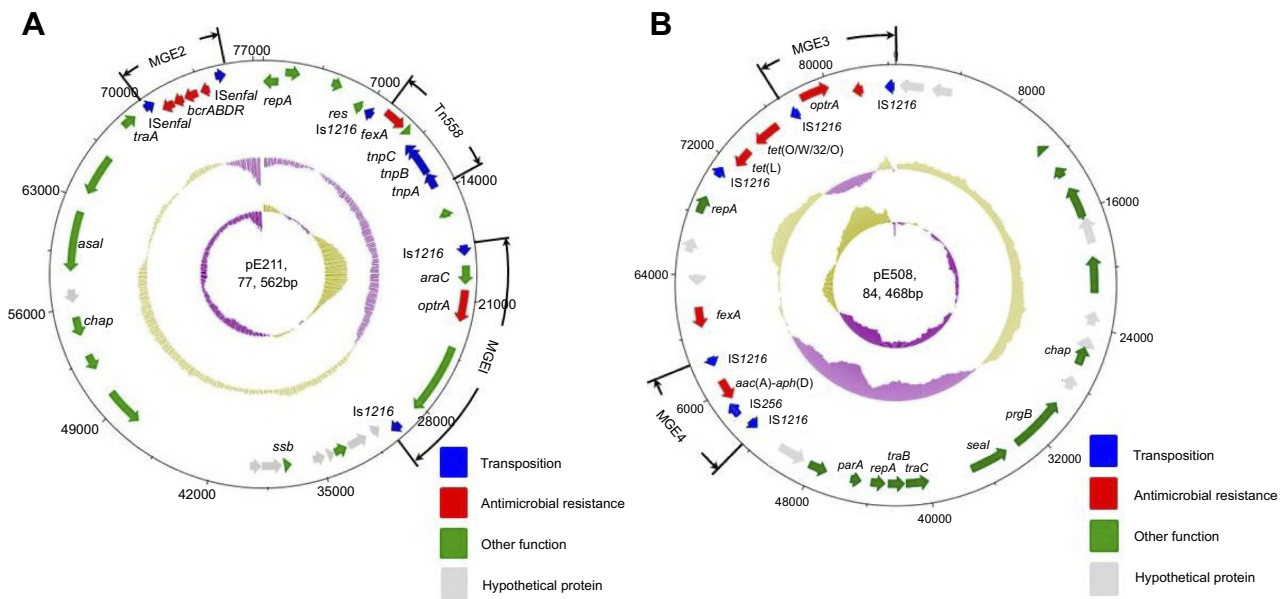


Figure 1 The structure of two pheromone-responsive conjugative multiresistant plasmids carrying a mobile *optrA* locus from *E. faecalis* in this study **(A)** The structure of the plasmid pE211. The positions of two mobile elements (MGE1 and MGE2), and Tn558 were indicated in bold vertical lines and arrows outside the plasmid, **(B)** The structure of the plasmid pE508. The positions of two mobile elements (MGE3 and MGE4) were indicated in bold vertical lines and arrows outside the plasmid. The circles display (from the outside to inside): (i) the size scale in bp; (ii) the positions of predicted coding sequences transcribed in the clockwise orientation; (iii) the positions of predicted coding sequences transcribed in the counterclockwise orientation; (iv) the GC content plotted against 50%, with orange indicating >50% and purple indicating <50%; and (v) GC skew [(G-C)/(G+C)] in a 10,000 bp window. Genes are colour-coded, depending on functional annotations: blue, transposition; red, antimicrobial resistance; green, other function; gray, hypothetical protein.

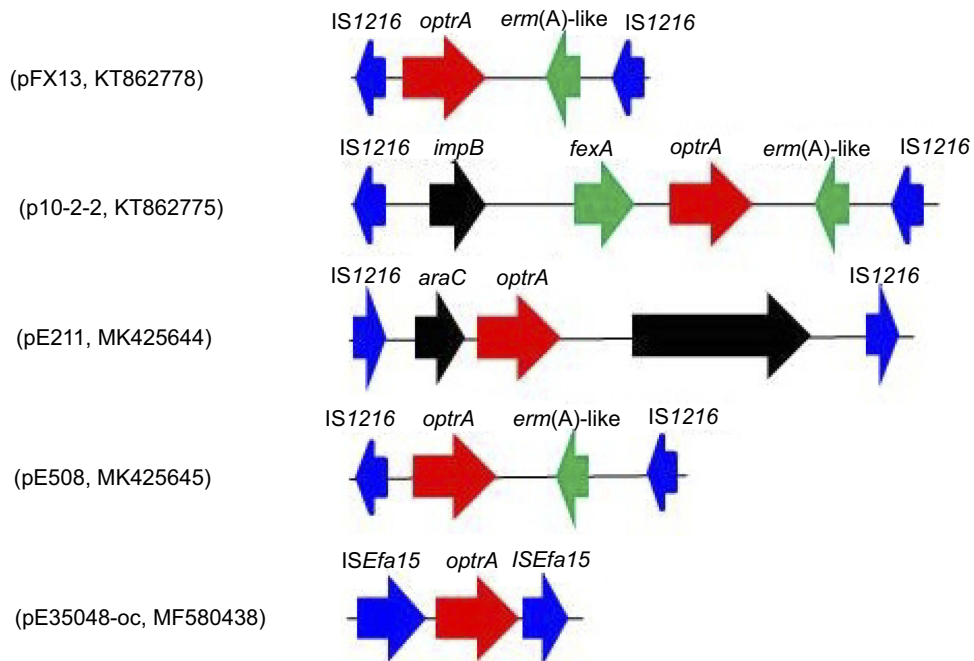


Figure 2 The environment of the *optrA* gene in different plasmids.

Table 4 Coding sequences of the plasmid pE2.1

CDS no.	CDS	Nucleotide position (5'→3')	Protein length (aa)	Database match (Size and accession no.)	aa identify (%)
1	repA	1-1008	335	replication initiator protein A, <i>Enterococcus faecalis</i> (335aa, WP_002382056.1)	99.7% (334/335)
2	orf	1358-2305	315	ATPase, <i>Enterococcus faecalis</i> (315aa, WP_025192929.1)	99.7% (314/315)
3	orf	4303-4971	222	CPBP family intramembrane metalloprotease, <i>Enterococcus faecalis</i> (222aa, WP_002403283.1)	99.5% (221/222)
4	orf	6038-6658	206	resolvase, N-terminal domain protein, <i>Enterococcus faecalis</i> (211aa, EJU10278.1)	97.6% (206/211)
5	ISI2/16	6830-7516	232	IS6-like element ISI2/16 family transposase, <i>Enterococcus faecium</i> (232aa, WP_014748744.1)	100.0% (232/232)
6	flexA	8026-9453	475aa	chloramphenicol/florfenicol efflux MFS transporter FlexA, <i>Enterococcus faecalis</i> (475aa, WP_078122474.1)	100.0% (475/475)
7	orf138	9631-10,047	138	putative oxidoreductase, <i>Staphylococcus saprophyticus</i> (138aa, AVE17237.1)	100.0% (138/138)
8	tnpC	10,330-10,695	121	Transposase C, <i>Staphylococcus aureus</i> (121aa, YP_007878373.1)	100.0% (121/121)
9	tnpB	10,697-12,616	639	Transposase B, <i>Staphylococcus cohnii</i> (639aa, AEP69225.1)	100.0% (639/639)
10	tnpA	12,613-13,698	361	Transposase A, <i>Staphylococcus epidermidis</i> (361aa, AJW29167.1)	100.0% (361/361)
11	orf	15,103-15,726	207	resolvase helix-turn-helix protein, <i>Enterococcus faecium</i> (207aa, ADO66759.1)	100.0% (207/207)
12	ISI2/16E	17,617-18,297	226	IS6-like element ISI2/16 family transposase, <i>Enterococcus faecium</i> (226aa, YP_006937527.1)	100.0% (226/226)
13	araC	18,886-20,040	384	AraC family transcriptional regulator, <i>Enterococcus faecalis</i> (384aa, AMM74624.1)	100.0% (383/384)
14	optrA	20,371-22,338	655	ABC-F type ribosomal protection protein Optra, <i>Enterococcus faecalis</i> (655aa, WP_078122475.1)	99.7% (653/655)
15	orf	23,967-28,367	1466	restriction endonuclease, Enterococcaceae bacterium (1466aa, QBA99712.1)	100.0% (1466/1466)
16	ISI2/16E	29,642-30,322	226	IS6-like element ISI2/16 family transposase, <i>Enterococcus faecium</i> (226aa, YP_006937527.1)	100.0% (226/226)
17	hp	30,735-31,376	213	Hypothetical protein, <i>Enterococcus faecalis</i> (213aa, AEF32577.1)	100.0% (213/213)
18	hp	31,758-33,086	442	Hypothetical protein, <i>Enterococcus faecalis</i> (442aa, AEF32578.1)	99.8% (441/442)
19	orf	33,195-34,076	293	DNA nuclease, <i>Enterococcus faecalis</i> (293aa, AEF32579.1)	100.0% (293/293)
20	hp	34,048-34,491	147	Hypothetical protein, <i>Enterococcus faecalis</i> (147aa, AEF32580.1)	100.0% (147/147)
21	hp	34,705-35,541	278	Hypothetical protein, <i>Enterococcus faecalis</i> (278aa, WP_127341853.1)	99.6% (277/278)
22	ssb	36,916-37,392	158	Single-strand binding protein, <i>Enterococcus faecalis</i> (158aa, NP_816947.1)	100.0% (158/158)
23	hp	37,532-38,872	446	Hypothetical protein, <i>Enterococcus faecalis</i> (446aa, WP_080008653.1)	99.6% (444/446)
24	hp	38,863-39,639	258	Hypothetical protein, <i>Enterococcus faecalis</i> (258aa, YP_004032980.1)	100.0% (258/258)
25	orf	47,434-49,998	846	Type VI secretion protein, <i>Enterococcus faecalis</i> (846aa, OIU90382.1)	99.8% (844/846)
26	orf	51,750-52,784	344	Conjugal transfer protein, <i>Enterococcus faecalis</i> (344aa, WP_002387763.1)	100.0% (344/344)
27	chap	54,111-55,382	423	CHAP domain-containing protein, <i>Enterococcus faecalis</i> (423aa, WP_010711028.1)	100.0% (423/423)
28	hp	56,288-57,166	292	Hypothetical protein, <i>Enterococcus faecalis</i> (292aa, NP_816968.1)	100.0% (292/292)
29	asaI	58,328-62,218	1296	aggregation substance, Enterococcaceae bacterium (1296aa, QBA99726.1)	100.0% (1296/1296)
30	orf	63,454-66,174	906	LPXTG-motif cell wall anchor domain protein, <i>Enterococcus faecalis</i> (906aa, EFM78589.1)	100.0% (906/906)
31	traA	68,199-69,158	319	Conjugal transfer protein TraA, <i>Enterococcus faecalis</i> (319aa, NP_816935.1)	100.0% (319/319)
32	ISEnfaI	69,902-70,582	226	ISEnfaI family transposase, <i>Staphylococcus aureus</i> (226aa, WP_000191454.1)	100.0% (226/226)
33	bcrD	70,779-71,609	276	Undecaprenyl-diphosphatase, <i>Enterococcus faecalis</i> (276aa, AOX48039.1)	100.0% (276/276)
34	bcrB	71,609-72,310	249	bacitracin ABC transporter permease, <i>Enterococcus faecalis</i> (249aa, WP_129343483.1)	100.0% (249/249)
35	bcrA	72,351-73,268	305	bacitracin ABC transporter, ATP-binding protein BcrA, <i>Enterococcus faecalis</i> (305aa, AQL55357.1)	100.0% (305/305)
36	bcrR	73,451-74,065	204	XRE family transcriptional regulator, <i>Enterococcus faecalis</i> (204aa, AXG90118.1)	100.0% (204/204)
37	ISEnfaI	74,621-75,301	226	ISEnfaI family transposase, <i>Staphylococcus aureus</i> (226aa, WP_000191454.1)	100.0% (226/226)

Abbreviations: hp, hypothetical protein; aa, amino acids.

Table 5 Coding sequences of plasmid pE508

CDS no.	CDS	Nucleotide position (5'→3')	Protein length (aa)	Database match (Size and accession no.)	aa identify (%)
1	hp	140–1600	486	Hypothetical protein, <i>Enterococcus faecalis</i> (486aa, WP_126254905.1)	100.0% (486/486)
2	hp	3025–3753	242	Hypothetical protein, <i>Enterococcus faecalis</i> (242aa, WP_126266300.1)	100.0% (242/242)
3	orf	10,688–11,188	166	DnaJ domain-containing protein, partial, <i>Enterococcus faecalis</i> (173aa, EOH11044.1)	95.4% (165/173)
4	orf	12,574–13,359	261	ArsR family transcriptional regulator, <i>Enterococcus faecalis</i> (261aa, WP_002387611.1)	100.0% (261/261)
5	hp	14,217–16,430	737	hypothetical protein, <i>Enterococcus faecalis</i> (737aa, ETJ10394.1)	99.3% (732/737)
6	hp	16,417–18,579	720	hypothetical protein, <i>Enterococcus faecalis</i> (720aa, WP_087548822.1)	100.0% (720/720)
7	orf	19,135–21,627	830	type VI secretion protein, <i>Enterococcus faecalis</i> (846aa, OIU90382.1)	98.1% (830/846)
8	orf	23,001–24,035	344	conjugal transfer protein, <i>Enterococcus faecalis</i> (344aa, WP_010774162.1)	99.7% (343/344)
9	hp	24,742–25,359	205	Hypothetical protein, <i>Enterococcus faecalis</i> (205aa, WP_033786897.1)	99.5% (204/205)
10	chap	25,362–26,633	423	CHAP domain protein, <i>Enterococcus faecalis</i> (423aa, EFU06796.1)	100.0% (423/423)
11	hp	27,539–28,417	292	Hypothetical protein, <i>Enterococcus faecalis</i> (292aa, WP_002405612.1)	100.0% (292/292)
12	prgB	29,580–33,497	1305	LPXTG cell wall anchor domain-containing protein, <i>Enterococcus faecalis</i> (1305aa, WP_010819058.1)	99.6% (1300/1305)
13	seal	34,280–37,003	907	Surface exclusion protein, Enterococcaceae bacterium (907aa, QBA99747.1)	100.0% (907/907)
14	traC	39,968–41,557	529	TraC protein, <i>Enterococcus faecalis</i> (529aa, EOK37046.1)	99.4% (526/529)
15	traB	41,607–42,764	385	TraB/GumN family protein, <i>Enterococcus faecalis</i> (385aa, WP_010717212.1)	100.0% (385/385)
16	repA2	42,934–43,944	336	replication initiator protein A, <i>Enterococcus faecalis</i> (336aa, WP_010774283.1)	100.0% (336/336)
17	parA	44,553–45,335	260	ParA family protein, <i>Enterococcus faecalis</i> (260aa, WP_010783395.1)	100.0% (260/260)
18	orf	46,984–48,300	438	Y-family DNA polymerase, <i>Enterococcus faecalis</i> (438aa, WP_126262290.1)	100.0% (438/438)
19	hp	48,617–50,647	669	Hypothetical protein, <i>Enterococcus faecalis</i> (669aa, WP_010829996.1)	99.2% (664/669)
20	IS1216	52,601–53,287	228	IS1216 family transposase, <i>Enterococcus faecalis</i> (228aa, WP_080114306.1)	100.0% (228/228)
21	IS256	53,912–55,084	390	IS256 transposase, <i>Staphylococcus aureus</i> (390aa, CAL22896.1)	99.7% (389/390)
22	aac(A)-aphI(D)	55,214–56,653	479	bifunctional aminoglycoside N-acetyltransferase/aminoglycoside phosphotransferase, <i>Staphylococcus cohnii</i> plasmid (479aa, YP_009090128.1)	100.0% (479/479)
23	IS1216	57,683–58,369	228	IS1216 family transposase, <i>Enterococcus faecalis</i> (228aa, WP_080114306.1)	100.0% (228/228)
24	flexA	60,449–61,876	475	Florfenicol/chloramphenicol exporter, <i>Staphylococcus lentus</i> (475aa, WP_032495681.1)	99.8% (474/475)
25	hp	63,385–63,990	201	Hypothetical protein, <i>Enterococcus faecalis</i> (201aa, OXC92628.1)	100.0% (201/201)
26	hp	65,562–66,395	277	GLY-Y1G nuclease family protein, <i>Lactococcus lactis</i> (277aa, WP_060416607.1)	99.6% (276/277)
27	repA	68,095–69,273	392	Replication initiator protein, Enterococcaceae bacterium (392aa, QBA99761.1)	100.0% (392/392)
28	IS1216	70,722–71,408	228	IS1216 family transposase, <i>Enterococcus faecalis</i> (228aa, WP_080114306.1)	100.0% (228/228)
29	tet(L)	71,967–73,343	458	tetracycline efflux MFS transporter Tet(L), <i>Streptococcus uberis</i> (458aa, WP_037627686.1)	99.8% (457/458)
30	tet (O/W/32/O)	74,006–75,925	639	tetracycline resistance ribosomal protection protein, <i>Streptococcus suis</i> (639aa, RRN51891.1)	100.0% (639/639)
31	IS1216	76,939–77,619	228	IS6-like element IS1216 family transposase, <i>Enterococcus faecalis</i> (228aa, WP_080114306.1)	100.0% (228/228)
32	optrA	78,123–80,090	655	ABC-F type ribosomal protection protein OptrA, <i>Lactobacillales</i> (655aa, WP_099809080.1)	99.8% (654/655)
33	erm(A)-like	81,507–82,238	242	23S rRNA (adenine(2058)-N(6)-methyltransferase Erm(A), <i>Lactobacillus salivarius</i> (242aa, WP_086201761.1)	100.0% (242/242)
34	IS1216	83,691–84,377	228	IS6-like element IS1216 family transposase, <i>Enterococcus faecalis</i> (228aa, WP_080114306.1)	100.0% (228/228)

Abbreviations: hp, hypothetical protein; aa, amino acids.

The analysis of the genetic context of *optrA* in plasmids pE211 and pE508

As shown in Figure 1A and Table 4, the 77,562 bp plasmid pE211 harbored the phenicol/oxazolidinone resistance gene *optrA*, the TN558-associated phenicol resistance gene *fexA*, and the mobile bacitracin resistance operon *bcrABDR* (Mobile Genetic Element, MGE2, 5,527 bp). The *fexA*-carrying transposon Tn558 has previously been described on plasmids in *Staphylococcus lentus*, *Staphylococcus cohnii*, and *Enterococcus* spp.^{11,18,19} Here, it is present on plasmid pE211 in *E. faecalis*. The MGE2 consisting of the *bcrABDR* operon confers resistance to bacitracin. The *bcr* locus was flanked by *ISEnfal* elements as previously described in *E. faecalis* or *Clostridium perfringens*.^{20,21} Here, it is present on the plasmid pE211 in *E. faecalis*.

As shown in Figure 1B and Table 5, the 84,468 bp plasmid pE508 harbored the phenicol/oxazolidinone resistance gene *optrA*, the phenicol resistance gene *fexA*, the mobile bifunctional aminoglycoside resistance gene *aac(A)-aph(D)* locus (MGE4, 5,891 bp), and the tetracycline resistance genes *tet* (L) and *tet*(O/W/32/O). The aminoglycoside resistance gene *aac(A)-aph(D)* is usually located on the transposon Tn4001 from staphylococci, Tn5281 from enterococci or Tn3706 from streptococci. Together with other resistance genes, it can also be located on the transposons Tn924, Tn5384 or Tn5385 from *E. faecalis*.²² In this study, to the best of our knowledge, it was for the first time seen that *aac(A)-aph(D)* is flanked by two copies of *IS1216* elements located in the same orientation on the plasmid pE508 from *E. faecalis*.

The presence of the circular intermediates in MGE2 and MGE4 were detected by inverse PCR (Table 2) and further sequence analysis indicated that both MGEs are active. However, the Tn558 locus is apparently not active as no circular intermediates were detectable.

Conclusion

Two pheromone-responsive conjugative multiresistance plasmids carrying the novel *optrA* locus from *E. faecalis* were identified, with one plasmid (pE211) harbouring a mobile *bcrABDR* locus, and the other (pE508) a mobile *aac(A)-aph(D)* locus. All these mobile locus were active due to the presence of the minicircles. The presence of MGEs in these plasmids renders them flexible and these elements will aid to the persistence and dissemination of these plasmids among enterococci and potentially also other Gram-positive bacteria.

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Disclosure

The authors report no conflicts of interest in this work.

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