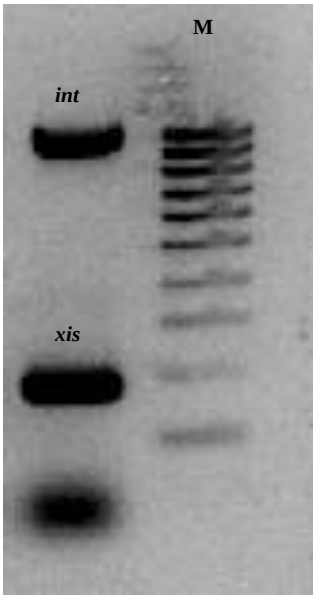
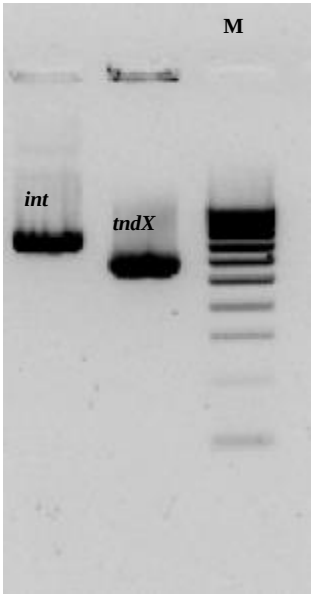
Table S1. PCR typing method for the detection of diverse Tn*916*-like conjugative transposons

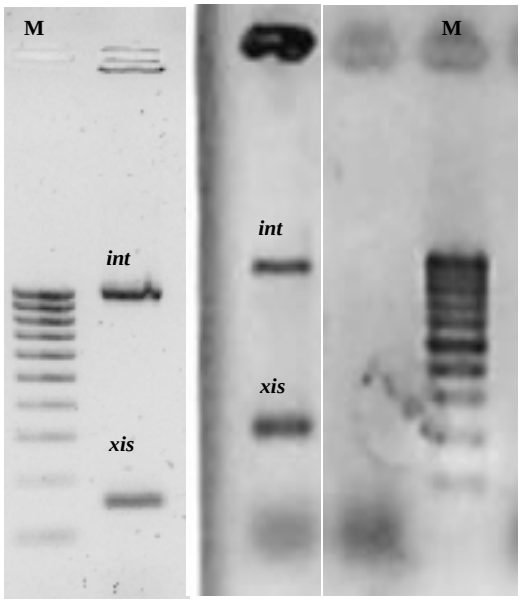
Multiplex 1
Tn*916*/Tn*1545*



Multiplex 2
Tn*5801* **Tn*5397***



Multiplex 3
Tn*5386* **Tn*6000***



PCR reaction	Primers	Sequence (5'-3')	Amplification conditions	Amplicon size (bp)	GenBank accession No.	Control isolates
Multiplex 1 Tn*916/1545*	Int_{916}-F	GCGTGATTGTATCTCACT	95ºC- 10'- 1 cycle; 94ºC- 30", 52ºC-30",72ºC-30"- 25 cycles; 72ºC-10'- 1 cycle	1046	U09422.1	*E. faecium* H638466 (this study)
	Int_{916}-R	GACGCTCCTGTTGCTTCT				
	Xis_{916}-F	AAGCAGACTGACATTCCTA		194		
	Xis_{916}-R	GCGTCCAATGTATCTATAA				
Multiplex 2 Tn*5397*	tndX-F	ATGATGGGTTGGACAAAGA	95ºC- 10'- 1 cycle; 94ºC- 30", 55ºC-30",72ºC-45"- 25 cycles;72ºC-10'- 1 cycle	611	AF333235	*E. faecium* H446511 (this study)
	tndX-R	CTTTGCTCGATAGGCTCTA				
Tn*5801*	Int_{5801}-F	CTGTTTCCGATATTGAGC		857	AF329848.1	*E. faecium* E240 (this study; KP001176.1)
	Int_{5801}-R	GTTTCGCAAGTAGTCTACAG				
Multiplex 3 Tn*6000*	Int_{6000}-F	CATCGAGTCTAACCGATTGT	95ºC- 10'- 1 cycle; 94ºC- 30", 55ºC-30",72ºC-30"- 25 cycles; 72ºC-10'- 1 cycle	869	FN555436.1	*E. faecalis* C386 (JN208881.1)
	Int_{6000}-R	GACCCAAACGAACTTGTACT				
	Xis_{6000}-F	CGAAGTATTAACCGAACAGA		203		
	Xis_{6000}-R	TATCATCGCGATCAAAACGA				
Tn*5386*	Int_{5386}-F	CTTGTTCCTACGGACAGAGT		1021	DQ321786.1	*E. faecium* DR344R[25]
	Int_{5386}-R	AGCCGTGAGCGTAATAATTC				
	Xis_{5386}-F	ATACTGATGTGCCTGTATGG		155		
	Xis_{5386}-R	TCGCTTTATCTGAATACGG				

Abbreviations: M, DNA marker- 100bp (Hypperladder IV 100bp-BioRon); *Int*, integrase; *xis*, excisionase, *tndX*, recombinase site-specific resolvase.

Table S3. Identification of specific nucleotide changes in each sequences with unique pattern, following the order of the phylogenetic consensus tree (Figure 2)**.

Variant	Species (number)	Country (variant)	Int_{Tn5801}	orf18	orf17	orf16	orf15	orf14	orf12	orf11	orf10	orf9	orf8	orf7	orf6	orf5	orf4
Type A																	
A 10	**Sau** (1)	CHN	T151C	-	-	A4479G	A5611G, T7199C	G7884A	-	T9310A	-	-	-	C12423T	-	-	-
A 10	**Sau** (1)	CHN	-	-	T2452G	A4479G	A5611G, T7199C	G7884A	-	T9310A	-	-	-	C12423T	-	-	-
A 10	**Sau** (2)	CHN	-	-	-	A4479G	A5611G, T7199C	G7884A	-	T9310A	-	-	-	C12423T	-	-	-
A 10	**Sau** (1)	unk	-	-	-	A4479G	T7199C	G7884A	-	Δ T8908, T9310A	-	-	-	C12423T	-	-	-
A 10	**Sau** (1)	BRA	-	-	-	-	T7199C	G7884A	-	T9310A	-	-	C10577A, T10723C	C12423T	-	-	-
A 10	**Sau** (1)	AUS	-	-	-	-	T7199C	G7884A	-	T9310A	-	-	C10032T	G11744A, C12423T	-	-	-
A 10	**Sau** (2)	AUS	T1085G	-	-	-	G6800A, T7199C	G7884A	-	T9310A	-	-	C10032T	C12066A, C12423T	G13254A	-	-
A 12	**Sau** (9)	USA	Δ381-478, A484C	-	-	-	D 5541-5549, C7199T	G7884A	-	T9310A	-	-	-	C12423T	-	-	-
A 11	**Sau** (1)	HKG	-	-	-	-	D 5541-5549, T7199C	G7884A	-	T9050C, T9310A	-	-	-	C12423T	-	-	-
A 10, A 11	**Sau** (13)	USA (A10), CHE (A10), HKG (A10), MYS (A11), CHN (A11), TWN (A11)	-	-	-	-	T7199C	G7884A	-	T9310A	-	-	-	C12423T	-	-	-
A 3	**Efm** (4)	FRA	-	-	Δ2237-2319	G3359A	-	-	-	-	-	-	T11131C	-	-	-	-
A 1	**Efm** (1)	GBR	-	-	G2636A	A3918T	A6574T	-	-	-	-	-	-	-	T13303C	-	-
A 1, A 4, A 5, A 6	**Sau** (3), **Efm** (9)	NLD (A4), FRA (A4, A6), PRT (A4), JPN (A1), AUS (A4, A5)	-	-	G2636A	A3918T	A6574T	-	-	-	-	-	-	-	T13303C	-	-
A 1	**Sps** (1)	unk	-	-	G2636A	A3918T	A6574T, CC6852TT	-	-	-	-	-	-	-	T13303C	-	A13764G
A 1	**Efm** (1)	DNK	-	-	T2529C, G2636A	A3918T	A6574T	-	-	-	-	-	-	-	T13303C	-	-
A 1	**Smi** (1)	DEU	-	-	G3046A	G3419A, T3586A, C4861T	-	-	-	-	-	-	-	-	T13303C	-	-
A 9	**Efm** (1)	unk	-	-	G3046A	T3586A, C4861T	-	-	-	-	-	-	G10586T	-	-	-	-
A 1	**Efs** (1)	DNK	-	-	G3046A	T3586A, C4861T	-	G7914A	-	-	-	-	-	G11506A	-	-	-
A 2, A 7	**Efm** (1), **Lga** (1)	IRL (A7), ESP (A2)	-	G1457A, T1804C	G3043C	-	G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 8, A 13	**Sau** (1), **Evi** (1)	USA (A13), (A8) (unk)	-	G1457A, T1804C	G2644A, G3043C	-	G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 1	**Sag** (1)	unk	-	G1457A, T1804C	A2928G, G3043C	IN: A4197	G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 1	**Sau** (1)	unk	ΔT1110	G1457A, T1804C	G3043C, T3084A	-	G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 14	**Sau** (1)	ESP	-	G1457A, T1804C	C2463T, G3043C	-	G7008T, IN: T 7374	-	-	-	-	-	-	-	C12895T	-	C13758T
A 1	**Sau** (86)	USA	-	G1457A, T1804C	G3043C	-	A5215T, G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 1	**Sau** (9)	USA	-	G1457A, T1804C, G2085A	G3043C	-	A5215T, G7008T	-	-	-	-	-	-	-	-	-	C13758T
A 1	**Sau** (6)	USA	-	G1457A, T1804C	G3043C	-	A5215T, G7008T	-	G8177A	-	-	-	-	-	-	-	C13758T
A 1	**Sau** (1)	unk	-	G1457A, T1804C	G3043C	G3267T	A5215T, G7008T	-	G8177A	-	-	-	-	C11910T	-	-	C13758T
Type B																	
B 18	**Efs** (2)	unk	A127G, A184G, A192G; C285T*, T402C*	-	A3046G*	A3586T*	T5575C	-	-	-	-	-	-	-	-	-	-

B 15	**Sag** (4)	GBR	-	G1420T	-	-	T6270C	-	Δ8070-8077, T8510C	-	-	-	-	-	-	-	-
B 20	**Sag** (1)	unk	-	-	-	-	-	-	Δ8070-8077	-	-	-	-	-	-	-	-
B 15, B 16, B 17, B 20	**Efm** (7), **Efs** (4), **Sag** (33), **Sth** (1)	USA (B20), GBR (B16), BEL (B16), ARG (B15), NOR (B15), unk (B17)	-	-	G3107T	-	-	-	Δ8070-8077	-	-	-	-	-	-	-	-
B 17	**Sag** (1)	unk	-	-	Δ2237-2319	-	-	-	Δ8070-8077	-	-	-	-	-	-	G13370A	-
B 20	**Sag** (1)	unk	-	-	-	-	-	-	-	-	-	-	-	ΔTC11143	-	-	A13905G
B 19	**Sag** (1)	unk	-	-	-	-	-	-	Δ8070-8077	T8953C	-	-	-	-	-	-	-
B 21	**Sag** (1)	USA	-	A1668C	Δ2237-2319	-	-	-	Δ8070-8077	-	-	-	A10355C	-	-	-	-
B 23	**Efs** (3)	GBR, FRA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B 23	**Efs** (2)	unk	-	-	-	-	-	-	-	-	-	-	-	A12056G	-	-	-
B 23	**Efs** (1)	unk	-	-	-	-	-	-	-	-	-	-	-	G12537C	-	-	-
B 23	**Efs** (2)	USA, GBR	-	-	-	-	C5514T	-	-	-	C9645T	-	-	C12525T	-	-	-
B 23	**Efs** (1)	USA	-	-	-	-	-	-	-	-	-	-	G10714T	-	-	-	-

** The position of each change is given from the alignment of the consensus sequences.

Δ, deletion; IN: insertion;

*These nucleotide changes are related to variants of type A (B18 is a recombinant variant).

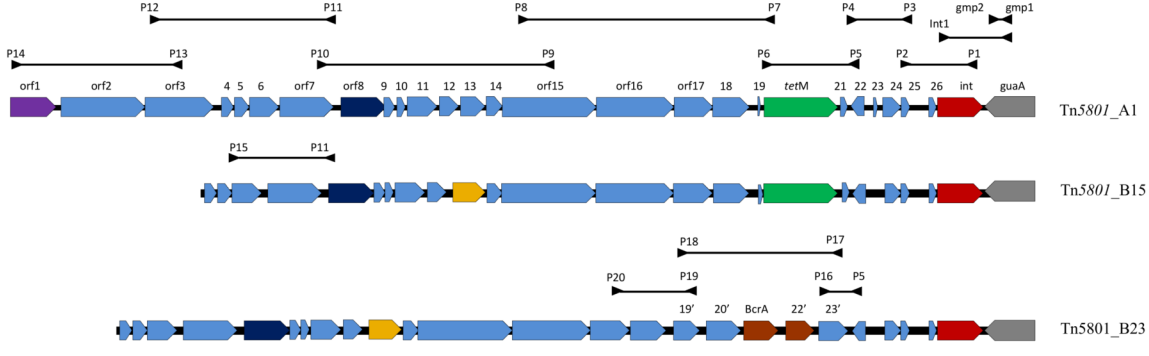

P12
P11
P8
P7
P4
P3
gmp2
gmp1
Int1
P14
P13
P10
P9
P6
P5
P2
P1
orf1
orf2
orf3
4
5
6
orf7
orf8
9
10
11
12
13
14
orf15
orf16
orf17
18
19
tetM
21
22
23
24
25
26
int
guaA
Tn5801_A1
P15
P11
Tn5801_B15
P18
P17
P20
P19
P16
P5
19'
20'
BcrA
22'
23'
Tn5801_B23

Figure S1

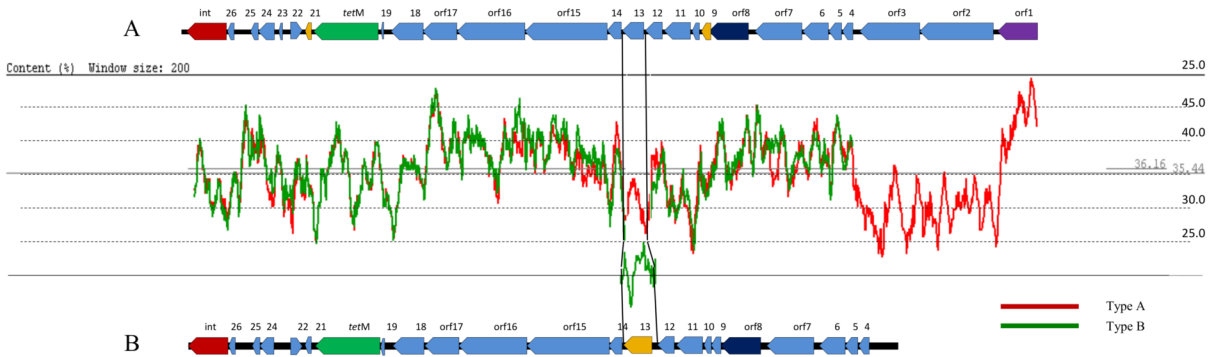

A
int 26 25 24 23 22 21 tetM 19 18 orf17 orf16 orf15 14 13 12 11 10 9 orf8 orf7 6 5 4 orf3 orf2 orf1
Content (%) Window size: 200
25.0
45.0
40.0
36.16
35.44
30.0
25.0
B
int 26 25 24 22 21 tetM 19 18 orf17 orf16 orf15 14 13 12 11 10 9 orf8 orf7 6 5 4
Type A
Type B

Figure S2

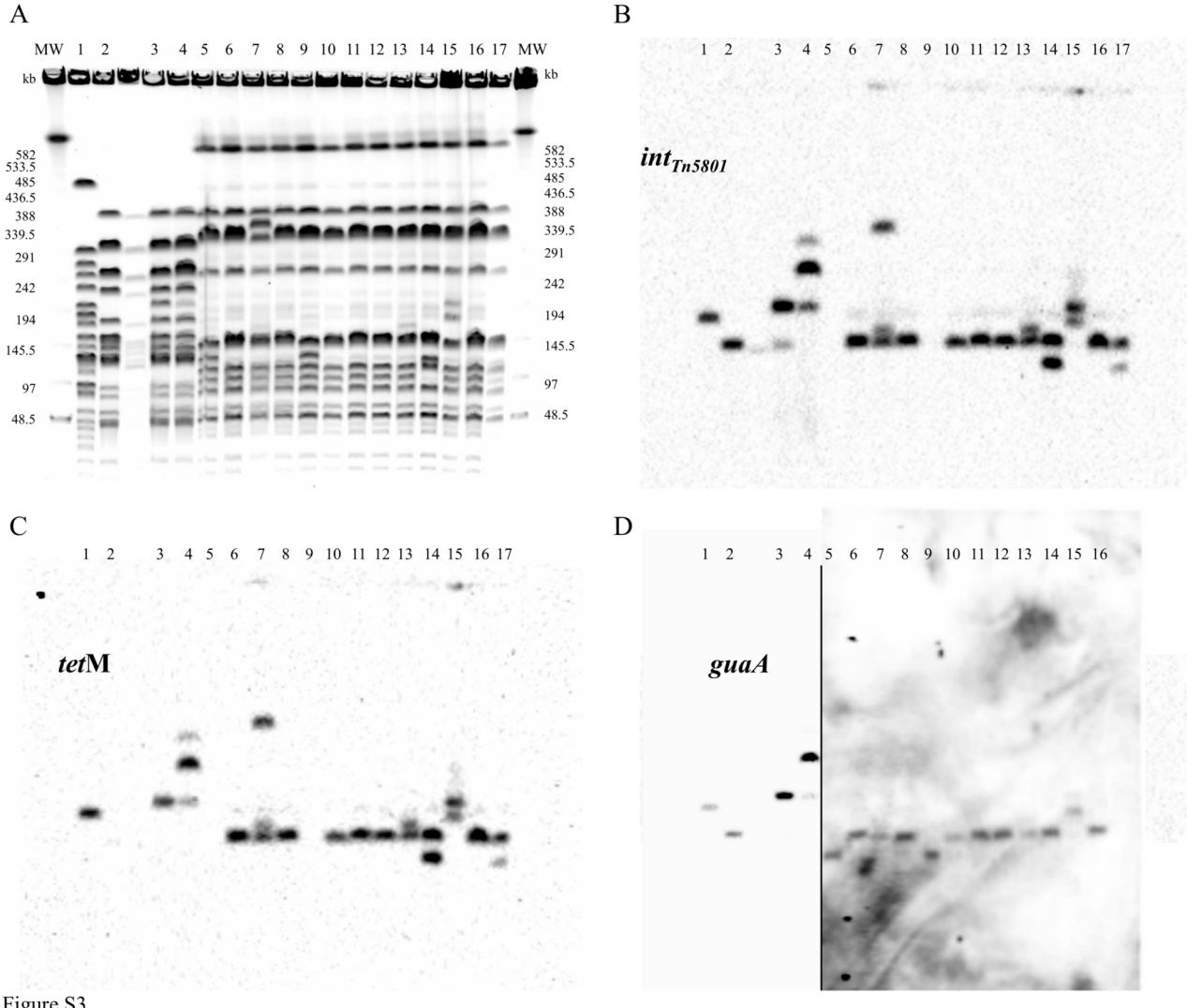

A
MW 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 MW
kb
582
533.5
485
436.5
388
339.5
291
242
194
145.5
97
48.5
B
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
int Tn5801
C
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
tetM
D
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
guaA

Figure S3

FIG S1. Characterization of Tn*5801*: PCR strategy.

FIG S2. GC% content of Tn*5801* A1 and B15.

FIG S3. Location of Tn*5801* markers int_{Tn5801}, *tet*(M) and *guaA* genes. *Sma*I-PFGE of *E. faecalis* donors, recipients and tranconjugant strains (A); hybridization of *Sma*I digested genomic *E. faecalis* DNA with a int_{Tn5801}, *tet*(M) and *guaA* probes (panels B, C, D, respectively). Primary transconjugants were represented in lines 3, 4, 10, 11 and 12; and secondary transconjugants in lines 6, 7, 8, 13, 14, 15, 16 and 17. MW= molecular weight marker (PFGE-standard lambda ladder, 48.5–1,000 kb; New England Biolabs); lane 1, Ef1; lane 2, *Efs* strain JH2-2; lane 3, *Efs* strain JH2-2::Tn*5801*_1; lane 4, *Efs* strain JH2-2::Tn*5801*_2; lane 5, *Efs* strain OG1SS; lane 6, *Efs* strain OG1SS::Tn*5801*_1.1; lane 7, *Efs* strain OG1SS::Tn*5801*_1.2; lane 8, *Efs* strain OG1SS::Tn*5801*_1.3; lane 9, *Efs* strain OG1RF; lane 10, *Efs* strain OG1RF::Tn*5801*_1; lane 11, *Efs* strain OG1RF::Tn*5801*_2; lane 12, *Efs* strain OG1RF::Tn*5801*_3; lane 13, Efs strain OG1SS::Tn*5801*_2.1; lane 14, *Efs* strain OG1SS::Tn*5801*_2.2; lane 15, *Efs* strain OG1SS::Tn*5801*_2.3; lane 16, *Efs* strain OG1SS::Tn*5801*_2.4; lane 17, *Efs* strain OG1SS::Tn*5801*_2.5. Panel D comprises two different hybridization experiments.