

Table S2. PCR primer pairs used in this study

Primers	Sequence (5'-3')	Genes	Product size (bp)	References
ESP14F	AGATTTTCATCTTTGATTCTTGG	<i>esp</i>	510	(1)
ESP12R	AATTGATTCTTTAGCATCTGG			
GEL11	TATGACAATGCTTTTTGGGAT	<i>gelE</i>	213	(2)
GEL12	AGATGCACCCGAAATAATATA			
HYLn1	ACAGAAGAGCTGCAGGAAATG	<i>hyl</i>	276	(2)
HYLn2	GACTGACGTCCAAGTTTCCAA			

Table S3. Comparison of *E. faecium* complete genomes

Strains	Chromosome / Plasmids	GC (%)	Length (bp)	CDS	rRNA	tRNA	GenBank	References
NRRL	Total	37.87	2,849,891	2,639	18	49	-	This study
B-2354	Chromosome	38.03	2,635,572	2,435	18	49	CP004063	
	Plasmid pNB2354_1	35.98	214,319	204	0	0	CP004064	
Aus0004	Total	38.31	3,019,780	2,932	18	47	-	(3)
	Chromosome	38.36	2,955,294	2,860	18	47	CP003351	
	Plasmid AUS0004_p1	35.39	56,520	63	0	0	CP003352	
	Plasmid AUS0004_p2	38.99	3,847	4	0	0	CP003353	
	Plasmid AUS0004_p3	36.81	4,119	5	0	0	CP003354	
TX16 (DO)	Total	37.87	3,052,572	3,114	18	64	-	(4)
	Chromosome	38.15	2,698,137	2,703	18	62	CP003583	
	Plasmid 1	36.51	36,262	43	0	0	CP003584	
	Plasmid 2	34.38	66,247	85	0	2	CP003585	
	Plasmid 3	35.97	251,926	283	0		CP003586	

Table S4. Enrichment of non-clinical (NC) and clinical (CL) isolates in MLST groups^a

MLST	No. of NC isolates	No. of CL isolates	Total No.	Origins enriched	<i>P</i> values
17	47	142	189	CL Isolates	2.51E-14
18	37	103	140	CL Isolates	4.28E-10
310	28	0	28	NC Isolates	3.86E-08
16	17	54	71	CL Isolates	7.67E-07
306	1	22	23	CL Isolates	1.10E-06
114	0	16	16	CL Isolates	5.19E-06
6	19	0	19	NC Isolates	7.82E-06
454	18	0	18	NC Isolates	1.46E-05
266	25	3	28	NC Isolates	8.19E-05
398	14	0	14	NC Isolates	1.83E-04
20	0	10	10	CL Isolates	4.89E-04
4	11	0	11	NC Isolates	1.26E-03
132	0	8	8	CL Isolates	2.24E-03
412	0	7	7	CL Isolates	4.79E-03
19	24	6	30	NC Isolates	4.79E-03
10	8	0	8	NC Isolates	8.89E-03
5	14	2	16	NC Isolates	9.41E-03
192	26	43	69	CL Isolates	1.01E-02
204	0	6	6	CL Isolates	1.02E-02
81	0	6	6	CL Isolates	1.02E-02
78	82	106	188	CL Isolates	1.15E-02
300	7	0	7	NC Isolates	1.72E-02
202	8	19	27	CL Isolates	1.84E-02
32	9	1	10	NC Isolates	2.45E-02
361	6	0	6	NC Isolates	3.35E-02
393	6	0	6	NC Isolates	3.35E-02
8	6	0	6	NC Isolates	3.35E-02
21	8	1	9	NC Isolates	4.31E-02
203	16	27	43	CL Isolates	4.37E-02
80	3	10	13	CL Isolates	4.68E-02
341	0	4	4	CL Isolates	4.71E-02

^a This table was summarized from the *E. faecium* MLST database (<http://efaecium.mlst.net/>) on May 30, 2012 and sorted by *P* values calculated by Fisher's Exact Test.

Table S5. Genes unique to NRRL B-2354

Clusters	Length (bp)	Products ^a	G+C (%)	Protein IDs ^b
SP1	3111	Type III restriction enzyme, DEAD/DEAH box helicase	38.96	AGE29399
SP1	129	Site-specific recombinase, phage integrase family	30.23	AGE29384
SP1	258	Hypothetical protein	33.72	AGE29392
SP1	1221	Hypothetical protein	31.70	AGE29386
SP1	597	Hypothetical protein	35.34	AGE29395
SP1	624	Hypothetical protein	33.33	AGE29389
SP1	1704	Phage integrase (Site-specific recombinase)	42.37	AGE29393
SP1	1335	Reverse transcriptase with group II intron origin	29.66	AGE29382
SP1	1839	Type III restriction-modification system methylation subunit	36.11	AGE29396
SP1	189	DNA repair protein RadC	37.57	AGE29387
SP1	366	Hypothetical protein	33.33	AGE29381
SP1	321	Hypothetical protein	36.14	AGE29388
SP1	1362	Hypothetical protein	41.63	AGE29400
SP1	198	Hypothetical protein	31.31	AGE29385
SP1	237	Putative DNA primase	33.76	AGE29391
SP1	831	Site-specific recombinase, phage integrase family	37.42	AGE29384
SP1	921	Hypothetical protein	33.55	AGE29390
SP1	924	Hypothetical protein	30.09	AGE29397
SP1	576	Hypothetical protein	31.60	AGE29398
SP1	1020	Restriction-modification system, specificity subunit	30.20	AGE29401
SP1	3189	DEAD/DEAH box helicase-like protein	37.06	AGE29394
SP1	438	DNA repair protein RadC	36.99	AGE29387
SP1	441	Hypothetical protein	36.51	AGE29383
SP2	162	Polysaccharide biosynthesis protein	25.47	AGE29666
SP2	411	Putative glycosyltransferase	30.90	AGE29664
SP2	132	Putative glycosyltransferase	31.06	AGE29668
SP2	147	Polysaccharide biosynthesis protein	24.66	AGE29666
SP3	120	Hypothetical protein	38.33	AGE29958
SP3	309	Hypothetical protein	39.81	AGE29964
SP3/SP5	402	Hypothetical protein	34.83	AGE29981
SP3/SP5	963	Bacteriophage mv4 main capsid protein Gp34	36.03	AGE29978
SP3/SP5	180	Hypothetical protein	33.89	AGE29979
SP3/SP5	351	Phage minor capsid protein	38.75	AGE29983
SP3/SP5	2253	Phage tail protein	39.59	AGE29992
SP3/SP5	390	Minor capsid protein	31.28	AGE29983

SP3/SP5	348	Phage minor capsid protein	32.76	AGE29982
SP3/SP5	360	Hypothetical protein	36.67	AGE29979
SP3/SP5	540	Minor capsid protein	38.70	AGE29985
SP3/SP5	408	Hypothetical protein	25.74	AGE30663
SP4	693	Transcriptional antiterminator of lichenan operon	28.14	AGE30148
SP4	1110	Transcriptional antiterminator of lichenan operon	29.37	AGE30148
SP4	1683	Amidohydrolase domain protein	31.97	AGE30150
SP4	1608	Major facilitator superfamily	35.45	AGE30149
SP5	489	Hypothetical protein	31.49	AGE30682
SP5	291	Hypothetical protein	29.55	AGE30683

^a Annotation was performed manually and included comparisons to 31 *E. faecium* genomes (5).

^b Protein IDs accession numbers in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>).

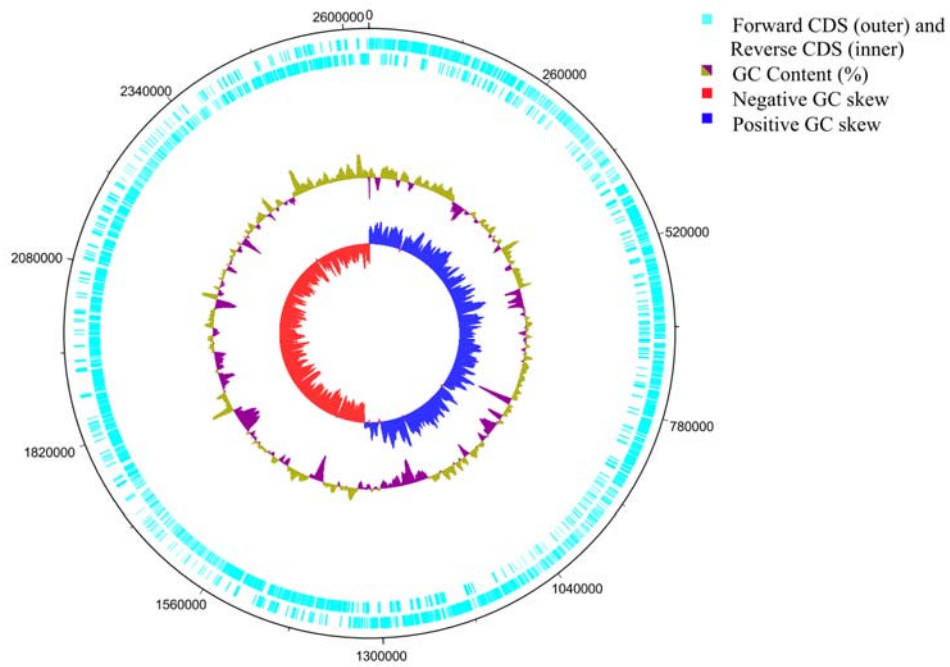


Figure S1. Circular map of the *E. faecium* NRRL B-2354 chromosome. The circular genome map was generated by using DNAPlotter (6). Tracks from inside to outside are as follows: GC skew $(G - C)/(G + C)$, GC content, reverse genes, and forward genes.

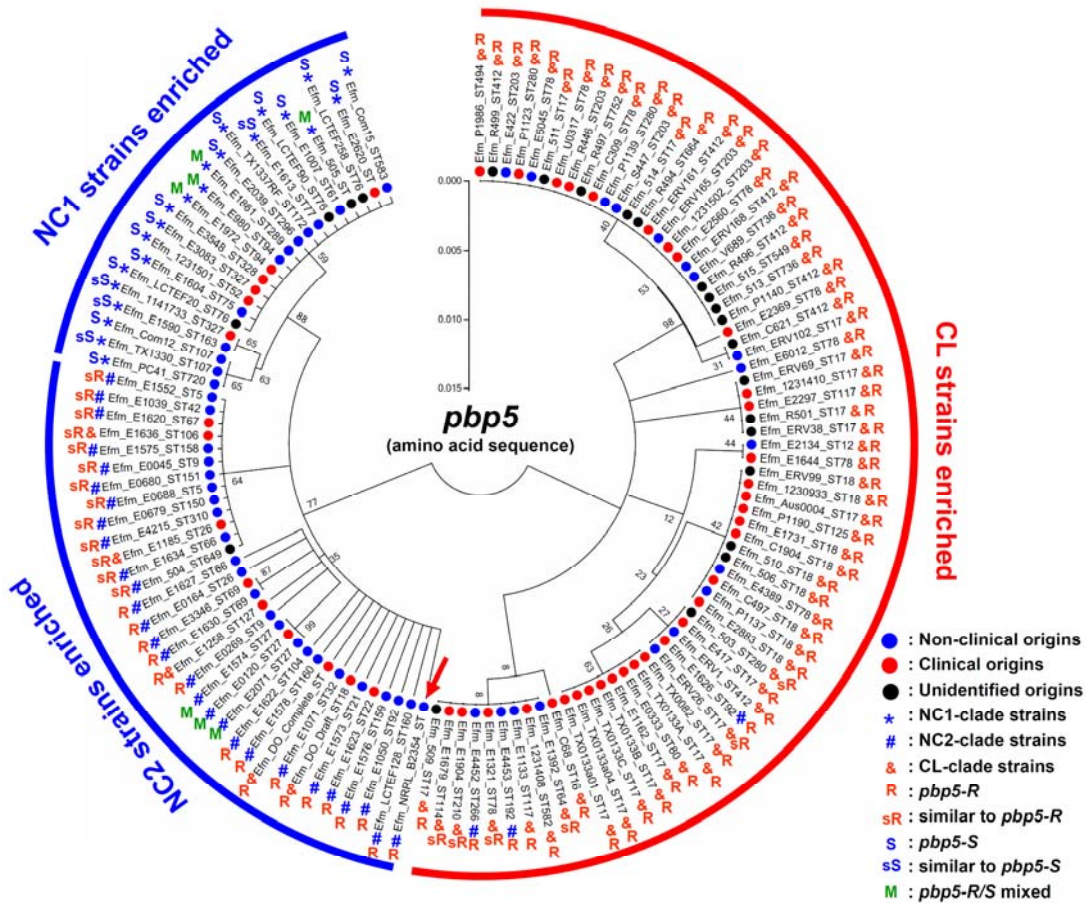


Figure S2. Phylogenetic tree of ampicillin-binding PBP5 amino acid sequences of 125 *E. faecium* genomes. Protein sequences of *pbp5* from 125 *E. faecium* genomes were analyzed to identify phylogenetic relationships. The PBP5 sequences from all strains shown in Figure 1 are included except for strains D344SRF and TC6 because the PBP5 gene was not identified in those genomes. *Pbp5* analysis was based on a previous study (7). The red arrow indicates the position of strain NRRL B-2354 in the dendrogram. The phylogenetic relationships were confirmed by 1,000 replicates of bootstrapping based on the neighbor joining method.

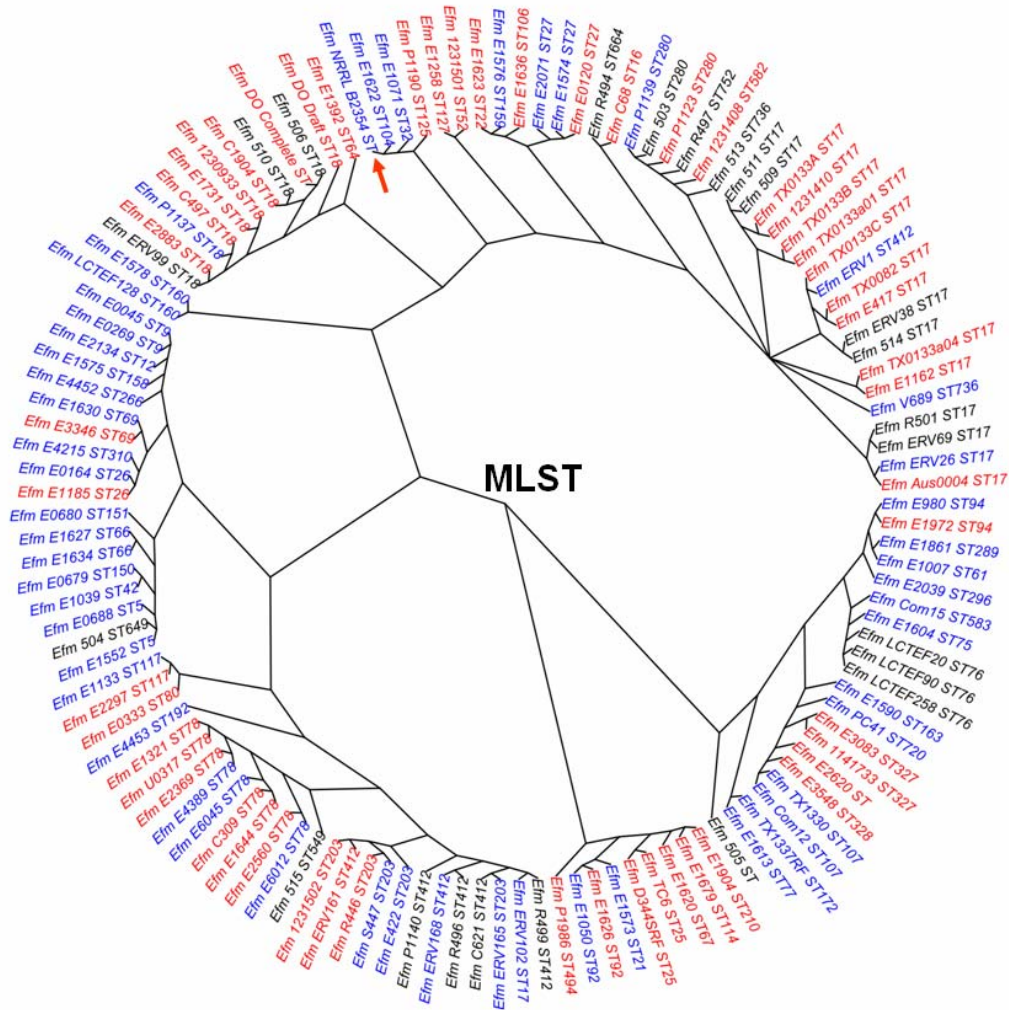


Figure S3. Phylogenetic tree of MLST sequences using 127 *E. faecium* strains.

Concatenated sequences of seven housekeeping genes (*adk*, *atpA*, *ddl*, *gdh*, *gyd*, *pstS*, and *purK*) from clinical (red), non-clinical (blue), and origin-unknown (black) strains were analyzed. NRRL B-2354 is indicated with a red arrow. The phylogenetic relationships were confirmed by 2,000 replicates of bootstrapping based on the neighbor joining method using MEGA5 (8).

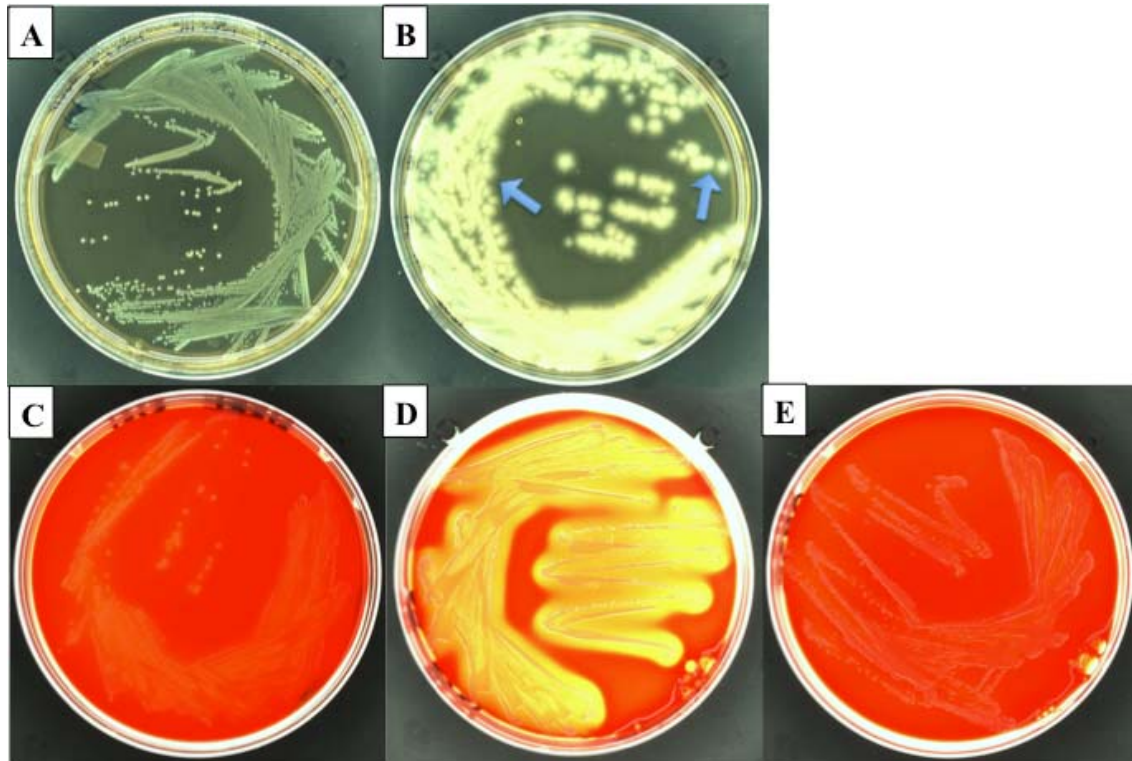


Figure S4. Gelatinase activity and hemolysin activity of NRRL B-2354. Growth of (A) *E. faecium* NRRL B-2354 and (B) positive control strain, *Bacillus cereus* ATCC 14579, on Todd Hewitt agar containing 3% gelatin. Arrows in (B) point to zones of turbidity, indicative of gelatin hydrolysis. Growth of (C) *E. faecium* NRRL B-2354, (D) the positive control strain, *E. faecalis* ATCC 29212, and (E) the negative control strain *E. coli* DH5 α on tryptic soy agar supplemented with 5% defibrinated horse blood. Zones of clearing in (D) indicate lysis of red blood cells.

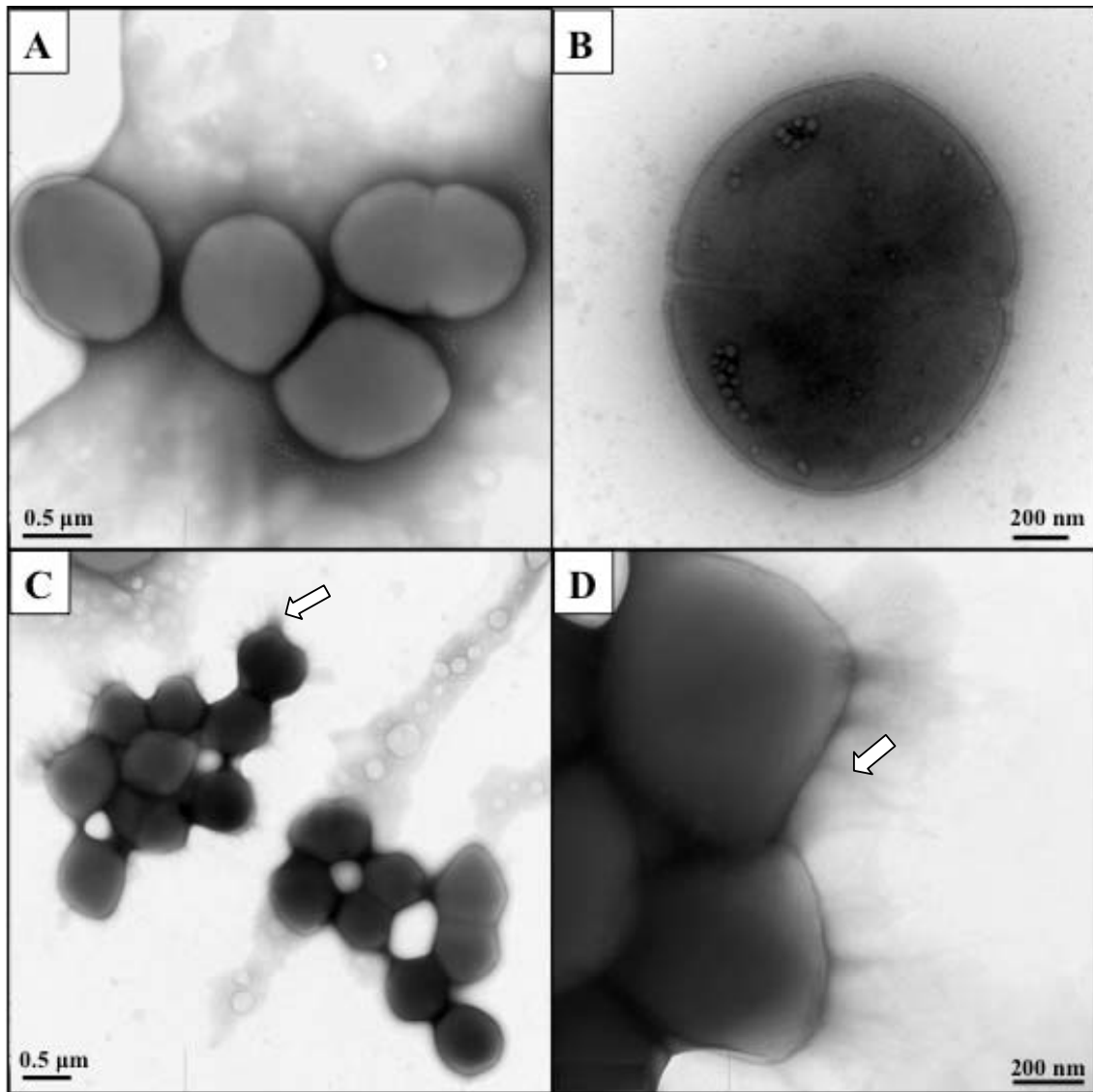


Figure S6. *E. faecium* pilus detection. (A-D) Transmission electron microscopy of *E. faecium* NRRL B-2354 and TX0082. (A-B) Pili were not found on *E. faecium* NRRL B-2354. (C-D) *E. faecium* TX0082 contained pili as indicated by the arrows.

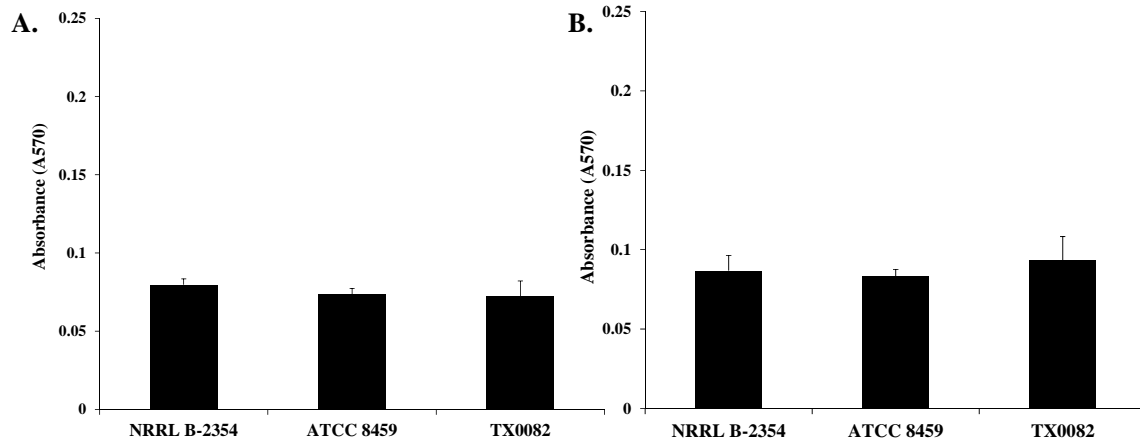


Figure S7. *E. faecium* binding to fibrinogen and fibronectin. (A) Binding to fibrinogen. No statistically significant difference was observed (Tukey's HSD, $P < 0.05$). The average \pm stdev of four replicates per strain is shown. (B) Binding to fibronectin. No statistically significant difference was observed (Tukey's HSD, $P < 0.05$). The average \pm stdev of four replicates per strain is shown.

References for Supplemental Materials

1. **Willems RJ, Homan W, Top J, van Santen-Verheувel M, Tribe D, Manzioros X, Gaillard C, Vandenbroucke-Grauls CM, Mascini EM, van Kregten E, van Embden JD, Bonten MJ.** 2001. Variant *esp* gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. *Lancet* **357**:853-855.
2. **Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H.** 2004. Development of a multiplex PCR for the detection of *asa1*, *gelE*, *cylA*, *esp*, and *hyl* genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *J. Clin. Microbiol.* **42**:4473-4479.
3. **Lam MM, Seemann T, Bulach DM, Gladman SL, Chen H, Haring V, Moore RJ, Ballard S, Grayson ML, Johnson PD, Howden BP, Stinear TP.** 2012. Comparative analysis of the first complete *Enterococcus faecium* genome. *J. Bacteriol.* **194**:2334-2341.
4. **Qin X, Galloway-Pena JR, Sillanpaa J, Hyeob Roh J, Nallapareddy SR, Chowdhury S, Bourgogne A, Choudhury T, Munzy DM, Buhay CJ, Ding Y, Dugan-Rocha S, Liu W, Kovar C, Sodergren E, Highlander S, Petrosino JF, Worley KC, Gibbs RA, Weinstock GM, Murray BE.** 2012. Complete genome sequence of *Enterococcus faecium* strain TX16 and comparative genomic analysis of *Enterococcus faecium* genomes. *BMC Microbiol.* **12**:135.
5. **Kim EB, Marco ML.** 2014. Non-clinical and clinical strains of *Enterococcus faecium* but not *Enterococcus faecalis* strains have distinct structural and functional genomic features. *Appl. Environ. Microbiol.* **80**(1):154-65.
6. **Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J.** 2009. DNAPlotter: circular and linear interactive genome visualization. *Bioinformatics* **25**:119-120
7. **Galloway-Peña JR, Rice LB, Murray BE.** 2011. Analysis of PBP5 of early U.S. isolates of *Enterococcus faecium*: sequence variation alone does not explain increasing ampicillin resistance over time. *Antimicrob. Agents Chemother.* **55**:3272-3277.
8. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**:2731-2739.