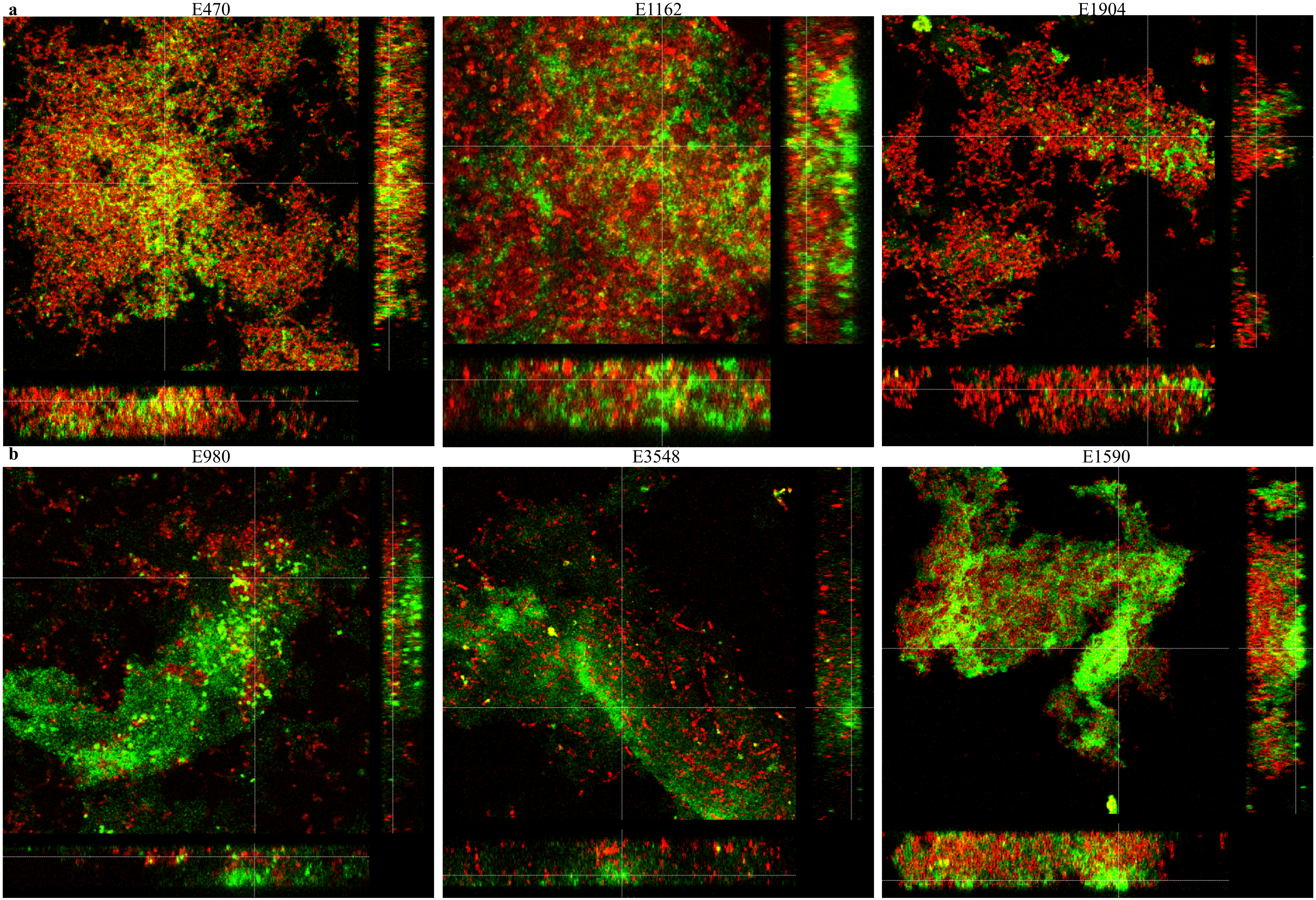

Figure S1. Confocal microscopy orthoview images depicting biofilm thickness and SagA localization in a semi-static biofilm model. Biofilms of three clade A1 (a) and three clade B strains (b), were grown for 24 h on poly-L-Lysine coated glass, in TSBg, at 120 rpm, at 37ºC. Biofilms were incubated with α-SagA and goat α-rabbit Alexa 488 (green). Bacterial membranes were stained with FM 95-5 (red). Pictures were taken at 63x magnification with 2.0 optical zoom.

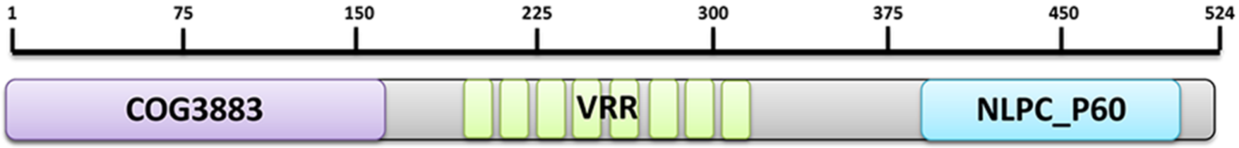

Figure S2. Prediction of structural domains of SagA protein sequence. COG3883 is an uncharacterized conserved domain identified in bacteria. NLPC_P60 is a conserved domain found in several lipoproteins involved in cell wall biogenesis. The central part of the protein, indicated with green bars, represents the variable repeat region (VRR) with differences between *E. faecium* strains belonging to clade A1 and B and the polyphyletic Intermediate Group (IG).

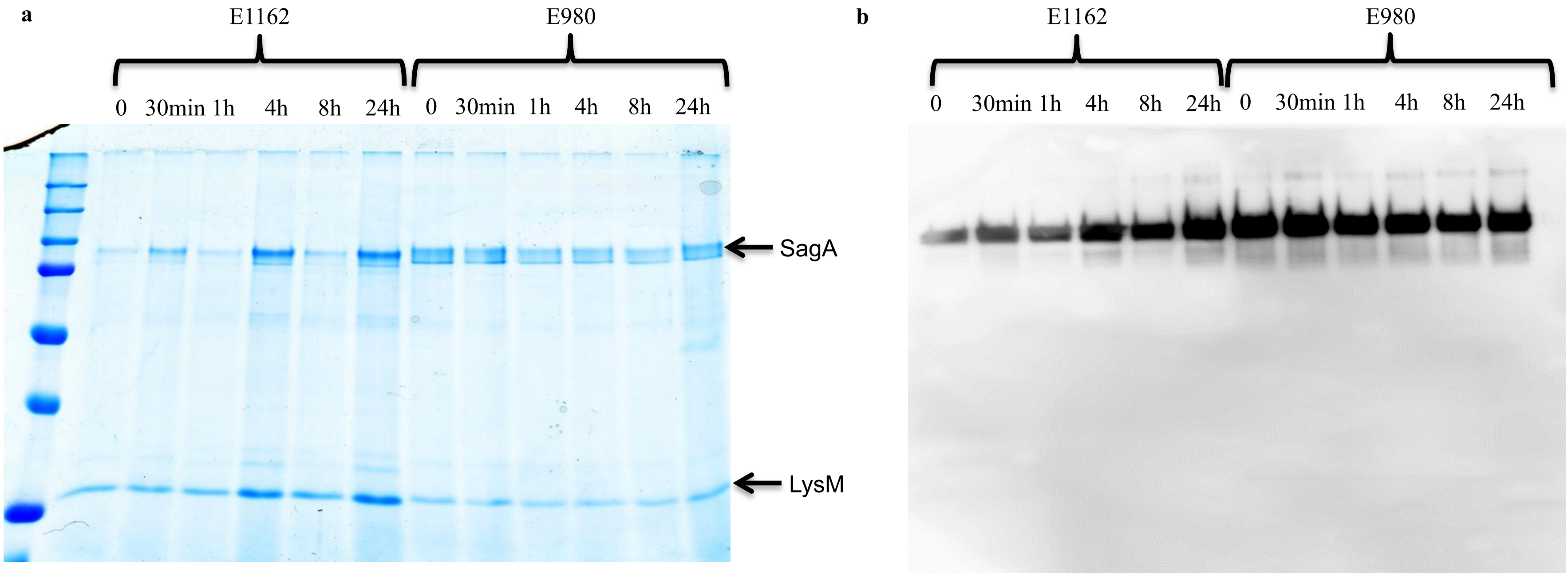

Figure S3. Stability of proteins released in the biofilm supernatant. a. Proteins present in the 24 h biofilm supernatant of hospital-associated (clade A1) *E. faecium* strain E1162 and community (clade B) *E. faecium* strain E980 were equally loaded and separated using a 12.5% SDS page gel and stained with Coomassie blue after incubation at 37ºC for 30 min, 1 h, 4 h, 8 h and 24 h. b. Presence of SagA in the biofilm supernatant of *E. faecium* strains after incubation at 37ºC, analyzed by Western Blot using rabbit α-SagA immune sera.

Figure S4. Phylogenetic tree based on the variable repeat region of SagA. SagA sequences from 85 *E. faecium* strains were used to build the phylogenic tree, based on amino acid level of selected variable repeat region by MUSCLE and Mega version 6. Numbers near branches indicate bootstrap values in percentage. Colored circles indicate the clade origin of strains according to core genome-based phylogeny. Clade A1 is indicated in red, IG is indicated in blue, and clade B is indicated in green.

Supporting data

Table S1. Ecological origin and clade assignment of 85 *E. faecium* strains.

Strains	Other name	Clade	ST	Country	Year	Epidemiology	Isolation site	Reference
E0045	EnGen0005	IG	9	GBR	1992	Poultry	Faeces	(1)
E120	EnGen0012	IG	27	NLD	1995	Clinical isolate	Ascites	(1)
E130	E0130	IG	6	NLD	1996	Community	Faeces	This study
E135	E0135	IG	6	NLD	1996	Community	Faeces	This study
E155	E0155	A1	17	USA	1995	Hospital outbreak	Faeces	(2)
E164	EnGen0010	IG	26	NLD	1996	Poultry	Faeces	(1)
E269	EnGen0022	IG	9	NLD	1996	Poultry	Faeces	(1)
E323	E0323	IG	79	FRA	1997	Clinical isolate	Faeces	This study
E333	EnGen0013	IG	80	ISR	1997	Clinical isolate	Blood	(1)
E470	E0470	IG	16	NLD	1999	Hospital outbreak	nd	This study
E679	EnGen0014	IG	150	BEL	1995	Pig	nd	(1)
E680	EnGen0019	IG	151	DEU	1995	Pig	nd	(1)
E688	EnGen0008	IG	5	ESP	1995	Pig	nd	(1)
E980	E0980	B	94	NLD	1998	Community	Faeces	(3)
E1007	EnGen0015	B	61	NLD	1998	Community	Faeces	(1)
E1028	E1028	IG	56	NLD	1998	Community	Faeces	This study

E1039	E1039	IG	42	NLD	1998	Community	Faeces	(3)
E1050	EnGen0017	A1	92	NLD	1998	Community	Faeces	(1)
E1071	E1071	A1	32	NLD	2000	Clinical isolate	Faeces	(3)
E1133	EnGen0002	A1	117	USA	2001	Clinical isolate	Faeces	(1)
E1141	E1141	IG	123	NLD	1995	Clinical isolate	nd	This study
E1162	E1162	A1	17	FRA	1997	Clinical isolate	Blood	(3)
E1172	E1172	A1	99	POL	1998	Clinical isolate	Urine	This study
E1185	EnGen0011	IG	26	FRA	1998	Clinical isolate	Blood	(1)
E1250	E1250	IG	25	CHE	1997	Clinical isolate	Blood	This study
E1258	EnGen0004	IG	127	ESP	1998	Clinical isolate	Blood	(1)
E1321	EnGen0054	A1	78	ITA	1999	Clinical isolate	Catheter	(1)
E1392	EnGen0016	A1	64	GBR	2000	Clinical isolate	nd	(1)
E1421	E1421	B	100	GBR	2000	Clinical isolate	nd	This study
E1435	E1435	A1	65	GRC	1999	Hospital outbreak	nd	This study
E1488	E1488	A1	32	ESP	2000	Community	Faeces	This study
E1552	EnGen0021	IG	5	NLD	2002	Clinical isolate	Faeces	(1)
E1573	EnGen0009	A1	21	BEL	1994	Bison	Rumen	(1)
E1574	EnGen0020	IG	27	BEL	1995	Dog	nd	(1)
E1575	EnGen0001	IG	158	BEL	1995	Poultry	nd	(1)

E1576	EnGen0018	A1	159	ZAF	2001	Ostrich	Ceacum	(1)
E1578	EnGen0007	A1	160	DEU	2001	Pig	Faeces	(1)
E1583	E1583	IG	161	BEL	2001	Community	Faeces	This study
E1590	EnGen0003	B	163	IRL	2001	Community	Faeces	(1)
E1604	EnGen0028	B	75	NOR	1956	Cheese	Food	(1)
E1613	EnGen0029	B	77	NOR	1964	Fish burger	Food	(1)
E1620	EnGen0027	IG	67	NLD	1957	Clinical isolate	Blood	(1)
E1622	EnGen0032	A1	104	NLD	1959	Rodent	nd	(1)
E1623	EnGen0031	A1	22	NLD	1960	Clinical isolate	Pus	(1)
E1626	EnGen0025	A1	92	NLD	1965	Clinical isolate	Stomach	(1)
E1627	EnGen0035	IG	66	NLD	1979	Clinical isolate	Gut	(1)
E1630	EnGen0039	IG	69	NLD	1981	Environment	Water	(1)
E1634	EnGen0040	IG	66	NLD	1982	Environment	Water	(1)
E1636	E1636	IG	106	NLD	1961	Clinical isolate	Blood	(3)
E1644	EnGen0051	A1	78	DEU	2002	Hospital outbreak	nd	(1)
E1669	E1669	B	94	BRA	nd	Clinical isolate	nd	This study
E1677	E1677	A1	113	BRA	nd	Clinical isolate	nd	This study
E1679	E1679	A1	114	BRA	1998	Hospital outbreak	Catheter	(3)
E1695	E1695	IG	97	BRA	1998	Clinical isolate	Faeces	This study

E1717	E1717	A1	18	DNK	nd	Hospital outbreak	nd	This study
E1721	E1721	A1	169	TZA	nd	Clinical isolate	Blood	This study
E1731	EnGen0036	A1	18	TZA	2002	Clinical isolate	Blood	(1)
E1760	E1760	A1	173	AUS	2000	Hospital outbreak	nd	This study
E1861	EnGen0042	B	289	ESP	2001	Clinical isolate	Faeces	(1)
E1904	EnGen0024	A1	210	NLD	2001	Clinical isolate	Urine	(1)
E1972	EnGen0033	B	299	DEU	2000	Clinical isolate	Blood	(1)
E2039	EnGen0026	B	296	DEU	2000	Clinical isolate	K-Spitze	(1)
E2071	EnGen0044	IG	27	DNK	2001	Poultry	nd	(1)
E2134	EnGen0043	IG	12	NLD	2004	Poultry	nd	(1)
E2297	EnGen0034	A1	117	USA	2001	Clinical isolate	Urine	(1)
E2369	EnGen0050	A1	78	HUN	2005	Clinical isolate	Wound	(1)
E2480	C68	A1	16	USA	1996	Hospital outbreak	nd	(1)
E2560	EnGen0046	A1	78	NLD	2006	Clinical isolate	Blood	(1)
E2620	EnGen0038	B	331	NLD	2006	Clinical isolate	Blood	(1)
E2883	EnGen0030	A1	325	NLD	2002	Clinical isolate	Blood	(1)
E3083	EnGen0056	B	327	NLD	2000	Clinical isolate	Blood	(1)
E3346	EnGen0052	IG	332	NLD	2002	Clinical isolate	Blood	(1)
E3548	EnGen0047	B	328	NLD	2004	Clinical isolate	Blood	(1)

E4215	EnGen0048	IG	310	SWE	2004	Poultry	nd	(1)
E4389	EnGen0057	A1	78	DNK	2008	Dog	Faeces	(1)
E4452	E4452	A1	266	NLD	2008	Dog	Faeces	(4)
E4453	E4453	A1	192	NLD	2008	Dog	Faeces	(4)
E6012	EnGen0045	A1	78	LVA	2010	Clinical isolate	Rectum	(1)
E6045	EnGen0049	A1	78	PRT	2010	Clinical isolate	Rectum	(1)
E7273	E7273	A1	117	ESP	2009	Clinical isolate	Faeces	This study
E7274	E7274	A1	203	ESP	2006	Clinical isolate	Blood	This study
E7275	E7275	IG	nd	ESP	2009	Clinical isolate	Faeces	This study
E7345	AUS0004	A1	17	AUS	1998	Clinical isolate	blood	(5)
E745	E745	A1	16	NLD	2000	Hospital outbreak	Faeces	unpublished
U0317	U0317	A1	78	NLD	2005	Clinical isolate	Urine	(3)

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Table S2. SNPs comparison between sequeced strains.

	E0045	E0120	E0130	E0135	E0155	E0164	E0269	E0323	E0333	E0470	E0679	E0680	E0688	E0980	E1007	E1028	E1039	E1050	E1071	E1133	E1141	E1162	E1172	E1185	E1250	E1258	E1321	E1392	E1421	E1435	E1488	E1552	E1573	E1574	E1575	E1576	E1578	E1583	E1590	E1604	E1613	E1620	E1622	E1623	E1626	E1627	E1630	E1634	E1636	E1644	E1669	E1677	E1679	E1695	E1717	E1721	E1731	E1760
E0045	0	6933	7873	6863	7775	2913	2047	4732	7308	5815	6936	6830	7000	45291	44859	4057	8443	6117	6699	10959	7721	7468	6750	5829	6013	8889	6346	7470	48939	7313	6498	7361	6518	6929	3376	6979	6628	12352	48920	44137	44547	2992	6309	6972	5989	7036	11602	6605	6262	6577	44850	6786	7228	6047	7214	8688	7219	7231
E0120	6933	0	7843	6833	5608	6782	7078	5040	5679	6568	7427	7138	6436	44544	44325	6015	8618	6074	5253	9421	7892	5300	5409	6310	6110	7129	5757	5518	47660	5753	5489	6843	5759	6	7791	4890	5263	11248	47634	43637	43953	7942	5322	4891	6039	5594	10565	6117	6008	5548	44307	6003	6366	6379	5396	7200	5625	5688
E0130	7873	7843	0	1044	7345	7287	7239	7621	6871	7866	4485	4642	3116	45903	45394	6350	6619	6143	7942	10546	7389	7051	6233	6729	6675	8554	7440	7078	49267	6866	6486	2099	6517	7839	7699	6442	5705	11655	49258	44727	45138	7637	6494	6462	6032	6323	11290	6307	6889	6724	45386	6816	6729	5314	6689	8110	6750	6754
E0135	6863	6833	1044	0	6328	6274	6229	6611	5856	6849	3462	3621	2091	45040	44529	5337	5598	5126	6924	9529	6373	6034	5213	5717	5663	7560	6427	6063	48393	5847	5469	1078	5504	6829	6688	5429	4688	10635	48384	43861	44276	6627	5481	5445	5014	5307	10269	5289	5877	5711	44523	5799	5717	4293	5670	7129	5731	5735
E0155	7775	5608	7345	6328	0	6849	7233	5228	1038	2328	6526	6330	7160	45099	44949	5481	7865	3148	4097	6066	7197	417	3151	5890	5755	6985	3523	1314	48341	964	2842	6462	3126	5604	7007	2473	2874	10160	48276	44249	44570	7349	2818	2393	3358	4786	10481	6186	5632	1686	45079	2910	5353	5550	1231	4113	1479	1920
E0164	2913	6782	7287	6274	6849	0	2271	4858	6373	4700	7010	6397	6791	46368	45880	4426	7914	6243	7026	10009	7765	6562	5708	4788	5115	7759	6711	6484	49645	6190	5894	6177	6152	6778	2685	5886	6049	11265	49639	45175	45572	3173	5860	5833	5915	5861	10934	6705	5368	6338	45883	6302	6704	5986	6260	7800	6316	6360
E0269	2047	7078	7239	6229	7233	2271	0	4677	6789	5379	6392	6202	6777	45363	44871	3367	7704	5563	6654	10399	7222	6946	6061	5468	5589	8262	6241	6895	49080	6725	5861	6610	5961	7074	3005	6316	5947	11870	49016	44162	44558	2446	5726	6283	5198	6331	11252	6607	5841	5921	44860	6172	6705	5422	6688	8175	6637	6776
E0323	4732	5040	7621	6611	5228	4858	4677	0	4962	6760	6800	6295	6170	44833	44491	3308	8092	5486	5390	8945	7432	5534	4834	4420	3902	7226	5196	4877	47945	5104	5261	6528	5319	5036	5743	4769	4802	10283	48025	43781	44180	5619	5122	4773	5311	4747	10079	5513	4273	5088	44493	5248	5728	5914	5162	6258	5052	5450
E0333	7308	5679	6871	5856	1038	6373	6789	4962	0	2941	6127	5899	6859	45340	44972	5032	7494	2733	4217	5578	6781	1021	2734	5384	5298	6806	3356	1191	48613	1342	2578	5921	2757	5675	6514	2207	2419	10211	48572	44274	44646	6930	2573	2167	2869	4296	10293	5732	5454	1217	44973	2395	5004	5094	766	3886	945	1670
E0470	5815	6568	7866	6849	2328	4700	5379	6760	2941	0	7618	7310	7681	46294	46100	6415	8517	4447	5378	7304	8155	1948	4266	6257	6646	7769	5371	3544	49524	2324	4141	6983	4425	6564	4924	3760	4173	11190	49464	45400	45723	5805	4119	3692	4650	5825	11178	7178	6484	3219	46228	4203	6434	6490	2852	5717	3191	3387
E0679	6936	7427	4485	3462	6526	7010	6392	6800	6127	7618	0	4999	5013	44840	44351	5438	5221	5314	7079	10016	7027	6564	5722	6787	6968	8197	7080	6270	48402	6274	5396	4070	5753	7423	6962	6225	5853	9945	48306	43686	44057	6681	5526	6168	5043	6403	10248	6725	7154	6177	44358	6217	6436	5110	6305	7845	6470	6644
E0680	6830	7138	4642	3621	6330	6397	6202	6295	5899	7310	4999	0	3977	44443	43926	5170	5340	5310	6666	9416	5681	6043	4979	4855	4841	7773	6771	6123	48091	6108	5244	3064	5284	7134	6461	5648	5574	11022	48044	43213	43662	6510	5242	5623	5195	4496	10740	2968	5064	6001	43932	5421	6088	3791	5688	7234	5690	5929
E0688	7000	6436	3116	2091	7160	6791	6777	6170	6859	7681	5013	3977	0	43936	43863	6709	6197	6385	6174	10602	7760	6866	6189	6245	6190	8564	6464	7058	47323	6651	6402	1333	6481	6432	7853	6392	6376	11664	47356	43377	43342	8160	6414	6403	6248	6331	10796	5023	6389	6492	43814	6706	7190	5970	6695	8206	6756	6772
E0980	45291	44544	45903	45040	45099	46368	45363	44833	45340	46294	44840	44443	43936	0	4381	44827	43847	44842	44837	42618	43307	45380	45775	45157	45123	46030	43804	45408	16175	45042	45378	44973	44928	44540	45739	45728	45404	40866	16074	3869	4308	45513	45313	45715	45033	45327	40742	44146	45206	45001	4419	45208	44694	44882	45619	44143	45517	45629
E1007	44859	44325	45394	44529	44949	45880	44871	44491	44972	46100	44351	43926	43863	4381	0	44468	43307	44440	44761	42212	42892	45178	45309	44688	44643	45604	43445	45072	17005	44828	44983	44422	44533	44321	45262	45350	45016	40391	16834	2662	4046	45040	44936	45353	44618	44926	40435	43680	44737	44604	417	44804	44292	44363	45222	43716	45110	45163
E1028	4057	6015	6350	5337	5481	4426	3367	3308	5032	6415	5438	5170	6709	44827	44468	0	6659	3791	5626	8749	5650	5179	4637	4411	3938	6986	5313	5187	48341	5470	4196	5947	4251	6011	4427	4616	4272	10753	48286	43752	44124	3610	3930	4594	3685	4642	10506	5316	4225	4685	44464	4539	5019	4106	4977	6635	4920	5241
E1039	8443	8618	6619	5598	7865	7914	7704	8092	7494	8517	5221	5340	6197	43847	43307	6659	0	6841	8352	10917	7854	7621	7250	6353	6600	8875	8288	7636	47593	7719	6844	5276	6887	8614	8078	7234	7292	8633	47515	42659	43102	7913	6579	7217	6712	7106	8246	6270	6804	7682	43310	7127	8176	6137	7395	8994	7349	7560
E1050	6117	6074	6143	5126	3148	6243	5563	5486	2733	4447	5314	5310	6385	44842	44440	3791	6841	0	3018	6827	5357	2847	3482	5768	5588	7102	3394	2487	48265	3328	1506	5568	1592	6070	6440	2066	1639	10715	48222	43837	44156	5748	1516	2038	1316	4375	10474	5115	5774	2420	44502	2050	4874	4066	2783	5640	3076	3436
E1071	6699	5253	7942	6924	4097	7026	6654	5390	4217	5378	7079	6666	6174	44837	44761	5626	8352	3018	0	8463	7272	3794	4853	7223	7030	7668	4025	3988	47265	4417	2270	6862	2724	5249	7750	2907	3232	11895	47266	44125	44258	7592	2500	2878	2948	5835	11361	5612	6963	4009	44786	3257	6461	5855	3900	6548	4217	4800
E1133	10959	9421	10546	9529	6066	10009	10399	8945	5578	7304	10016	9416	10602	42618	42212	8749	10917	6827	8463	0	10406	5681	6723	8589	8588	10166	7596	5946	44450	6520	6888	9674	6918	9417	10534	6451	6514	13932	44531	41489	41883	10548	6811	6390	7028	7778	13693	9189	8987	5873	42213	7004	8959	8738	5553	8837	5888	6189
E1141	7721	7892	7389	6373	7197	7765	7222	7432	6781	8155	7027	5681	7760	43307	42892	5650	7854	5357	7272	10406	0	6900	6262	7233	7236	8595	7215	6823	47031	7205	5840	6828	5928	7888	7773	6371	5887	9126	46960	42207	42816	7659	5768	6303	5447	4731	8904	4848	7420	6531	42895	6178	6410	2753	6640	8085	6557	6849
E1162	7468	5300	7051	6034	417	6562	6946	5534	1021	1948	6564	6043	6866	45380	45178	5179	7621	2847	3794	5681	6900	0	2888	5592	5505	6692	3453	1619	48613	1224	2541	6168	2825	5296	6710	2172	2573	10389	48552	44482	44803	7046	2519	2092	3050	4489	10378	5883	5382	1303	45308	2609	5100	5240	941	4420	1273	1765
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E1185	5829	6310	6729	5717	5890	4788	5468	4420	5384	6257	6787	4855	6245	45157	44688	4411	6353	5768	7223	8589	7233	5592	4894	0	994	6996	6850	5572	48608	5671	5726	5288	5760	6306	5427	5225	5304	9989	48591	43967	44428	5911	5562	5180	5771	4381	9885	4982	1450	5729	44688	5651	5396	5762	5237	7101	5210	5400
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E2480	5809	6560	7871	6854	2304	4736	5418	6759	2907	92	7623	7321	7685	46300	46116	6399	8516	4420	5351	7266	8166	1912	4228	6267	6623	7754	5341	3498	49536	2292	4109	6988	4398	6556	4918	3726	4146	11179	49476	45410	45728	5811	4087	3665	4618	5820	11163	7157	6494	3195	46239	4178	6445	6497	2820	5685	3159	3364
E2560	6830	5842	7771	6754	3199	7289	6743	5048	3065	5391	6882	7152	6790	43693	43347	5704	8698	3245	4019	7601	7545	3490	4604	7171	7040	8338	1500	2827	47103	3649	3762	7136	3967	5838	8342	3795	3437	11621	47065	42651	43061	7785	3886	3777	3496	5884	11035	6326	7238	2552	43337	4025	6248	6126	3471	6285	3696	4144
E2620	48149	47020	48540	47669	47562	48980	48318	47434	47862	48802	47551	47251	46604	14869	15318	47516	46779	47424	47140	44414	46206	47837	48405	47877	47914	48298	46532	48024	5051	47602	47958	47600	47532	47016	48561	48280	47957	45263	4846	16590	15596	48394	47877	48261	47596	48072	45186	46903	48002	47529	15386	47836	47436	47663	48138	46861	48094	48133
E2883	7169	5539	6676	5661	1785	6212	6617	5372	1254	3016	6732	5864	6687	45594	45165	5096	7465	3235	4366	5937	6694	1423	2748	5407	5077	6632	3620	1948	48839	2108	2784	5769	3158	5535	6317	2417	2724	10587	48806	44460	44856	6845	2827	2384	3199	4338	10539	5750	5048	1798	45170	2732	4836	4972	681	3206	559	951
E3083	49225	47925	49627	48694	48574	49889	49311	48265	48839	49763	48665	48321	47672	16681	17593	48594	47858	48533	47515	44824	47319	48848	49316	48825	48827	48831	47579	48985	2765	48592	49014	48605	48574	47921	49639	49237	48949	46105	2754	18522	17335	49443	48914	49227	48678	49025	46096	47990	48912	48557	17677	48889	48414	48756	49110	47813	49084	49084
E3346	11880	10912	11634	10613	10487	11282	11509	10083	10303	11516	9872	11000	11139	40348	40013	10854	8522	10816	11703	14035	9246	10716	10461	10225	10681	11680	11299	10050	45557	10299	10789	10678	10823	10908	12195	10276	10460	2840	45560	39275	39850	12253	10650	10283	10848	10168	454	11543	10925	10458	40017	10755	11296	10832	10624	11927	10596	10794
E3548	49378	48088	49733	48877	48761	50047	49475	48412	49035	49951	48772	48484	47823	16592	17389	48756	47935	48724	47701	45020	47456	49039	49483	49002	48991	49013	47726	49183	2876	48780	49210	48777	48778	48084	49797	49448	49161	46252	2295	18410	17181	49604	49119	49433	48893	49202	46225	48147	49082	48751	17487	49054	48633	48907	49322	47981	49296	49244
E4215	3024	7296	7237	6227	7094	1639	2930	5343	6688	5084	7207	6177	7000	46166	45652	4565	7891	6494	7471	10400	8188	6805	6027	4625	4885	8233	7439	6925	49526	6601	6174	6283	6422	7292	2804	6422	6571	11746	49546	44940	45353	3090	6090	6342	6283	6296	11503	6492	5140	6738	45657	6569	7011	6340	6594	8058	6592	6601
E4389	6625	5859	6329	5312	1603	6112	5980	5659	1873	3166	5662	5632	6502	45089	44894	4339	7258	2033	3710	5970	6076	1297	3026	5694	5624	7070	2446	2076	48328	2314	2567	5805	2609	5855	6875	2644	2360	10688	48266	44197	44513	6255	2522	2583	2205	4598	10470	5618	5788	1533	45019	3059	4735	4509	1814	5278	2251	2603
E4452	5273	5330	6649	5635	3854	5529	4990	4296	3452	5428	6266	5815	5607	44506	44088	4266	7655	3138	3414	7626	6488	3570	3890	5886	5814	7486	2037	3690	47945	4261	3366	6171	3412	5326	6834	3324	2976	11093	47914	43402	43794	6296	3410	3318	2785	5111	10474	5242	6047	2542	44137	3642	5077	4862	3327	6116	3533	4035
E4453	6645	5604	6428	5401	1959	5979	5884	5076	1481	3526	5837	5790	6575	45184	44789	4383	7470	2562	4016	6165	6240	1657	3023	5595	5515	7015	2982	2293	48461	2328	2699	6000	2860	5600	6547	2316	2014	10636	48405	44093	44476	6437	2743	2303	2194	4522	10230	5854	5731	919	44781	2422	4537	4617	1257	4572	1732	2348
E6012	6642	5959	6204	5186	2148	6014	5950	4801	1866	4354	5422	5715	6648	45024	44669	4407	7382	2239	3914	6471	6114	2443	3319	5856	5716	7326	2746	1756	48296	2532	2445	5352	2470	5955	6884	2470	1992	10403	48261	43979	44350	6346	2481	2428	2107	4659	10420	5533	5987	1971	44676	2856	4793	4648	2162	4815	2386	3028
E6045	6640	5943	6198	5184	2162	6012	5948	4795	1866	4356	5420	5707	6646	45014	44661	4407	7380	2247	3922	6485	6112	2457	3333	5854	5724	7330	2742	1766	48289	2546	2449	5350	2460	5939	6882	2460	1996	10401	48254	43972	44343	6344	2475	2432	2115	4657	10418	5531	5985	1971	44668	2864	4787	4646	2178	4827	2402	3044
E7273	6981	5188	7355	6338	1674	6608	6676	4668	2104	3863	6632	6357	6341	44425	44282	5441	8003	3186	3380	6555	7264	1964	3489	6274	6147	7537	2472	1976	47778	2461	3129	6489	3276	5184	7544	3177	3315	10717	47721	43575	43902	7491	3141	3156	3406	5120	10260	5684	6288	2183	44407	3728	5741	5667	2431	5099	2518	3130
E7274	6693	5883	7672	6645	3063	7372	6818	5048	2951	5257	6821	7080	6794	43675	43323	5615	8633	3378	4164	7471	7583	3360	4494	7101	6970	8428	1331	2934	47078	3536	3964	7211	4011	5879	8277	3978	3652	11654	47042	42622	43046	7681	3909	3989	3555	6060	11068	6239	7168	2410	43308	4364	6138	5999	3335	6137	3559	3974
E7275	9416	7912	6817	5797	6949	8134	8699	7696	6921	7946	6122	6388	6362	44166	43738	7696	3845	7659	8675	10608	8542	7048	6695	6408	6574	8528	8430	6678	47675	6853	7170	5281	7434	7908	8771	6267	6906	8947	47622	43058	43507	8939	7109	6175	7517	6466	8883	6302	6315	7476	43735	7653	7596	6635	6843	7912	6898	6955
E7345	7670	5499	7249	6232	263	6764	7147	5392	893	2185	6438	6243	7063	45231	45051	5385	7824	3055	4002	5911	7102	262	3100	5797	5721	6891	3664	1453	48477	1087	2744	6366	3031	5495	6908	2373	2781	10263	48409	44352	44670	7244	2718	2300	3253	4691	10579	6081	5589	1545	45176	2817	5313	5444	1092	4246	1333	1969
E745	5828	6587	7851	6834	2473	4716	5432	6792	2917	257	7490	7325	7701	46315	46121	6402	8528	4404	5373	7260	8132	2081	4069	6261	6617	7788	5368	3521	49563	2449	3944	6980	4382	6583	4909	3725	4132	11169	49515	45413	45751	5821	4089	3671	4513	5831	11168	7190	6484	3145	46246	4188	6312	6503	2812	5671	3151	3376
U0317	6826	5838	7767	6750	3195	7285	6739	5044	3061	5387	6878	7148	6786	43691	43345	5700	8694	3241	4015	7599	7541	3486	4600	7167	7036	8334	1496	2823	47101	3645	3758	7132	3963	5834	8338	3791	3433	11617	47063	42649	43059	7781	3882	3773	3492	5880	11031	6322	7234	2548	43335	4021	6244	6122	3467	6281	3692	4140

Table S3. Proteomics analysis of biofilm supernatant. Mass spectrometry of the excised protein bands from 24 h biofilm supernatant. Mass spectrometry was performed by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry, using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker Daltonics).

Band	**Accession**	**Locus**	**Mass**	**Score**	**Description**
75kDa	gi:424797365	ZP_18222963.1	51731	125	NlpC/P60 family protein [*Enterococcus faecium* S447]
	gi:314938222	ZP_07845522.1	52950	123	NlpC/P60 family protein [*Enterococcus faecium* TX0133a04]
	gi:314992106	ZP_07857556.1	52922	123	NlpC/P60 family protein [*Enterococcus faecium* TX0133B]
	gi:424790089	ZP_18216682.1	52977	123	NlpC/P60 family protein [*Enterococcus faecium* V689]
	gi:431747447	ZP_19536241.1	53108	123	protein P54 [*Enterococcus faecium* E2134]
	gi:289565032	ZP_06445486.1	53894	122	secreted antigen SagA [*Enterococcus faecium* D344SRF]
	gi:294623697	ZP_06702530.1	54140	122	SagA [*Enterococcus faecium* U0317]
	gi:383329927	YP_005355811.1	54411	122	NlpC/P60 family lipoprotein [*Enterococcus faecium* Aus0004]
50kDa	gi:69246939	ZP_00604194.1	80399	74	Sulfatase [*Enterococcus faecium* DO]
	gi:257878586	ZP_05658239.1	80411	74	sulfatase [*Enterococcus faecium* 1,230,933]
	gi:430835628	ZP_19453617.1	80415	74	sulfatase [*Enterococcus faecium* E0680]
	gi:415894466	ZP_11550330.1	71547	57	sulfatase domain protein [*Enterococcus faecium* E4453]
	gi:227551868	ZP_03981917.1	80308	56	phosphatidylglycerol--membrane-oligosaccharide glycerophosphotransferase [*Enterococcus faecium* TX1330]
	gi:257884281	ZP_05663934.1	80330	56	sulfatase [*Enterococcus faecium* 1,231,501]
25kDa	gi:69244843	ZP_00603067.1	21591	96	Peptidoglycan-binding LysM [*Enterococcus faecium* DO]
	gi:261208113	ZP_05922788.1	21633	96	peptidoglycan-binding LysM [*Enterococcus faecium* TC 6]
	gi:314941298	ZP_07848192.1	21621	96	LysM domain protein [*Enterococcus faecium* TX0133C]
	gi:424856196	ZP_18280450.1	21619	96	LysM domain protein [*Enterococcus faecium* R499]
	gi:430827476	ZP_19445618.1	21647	96	LysM protein [*Enterococcus faecium* E0164]
	gi:431303160	ZP_19508007.1	21018	69	LysM protein [*Enterococcus faecium* E1626]