

SUPPLEMENTAL MATERIALS

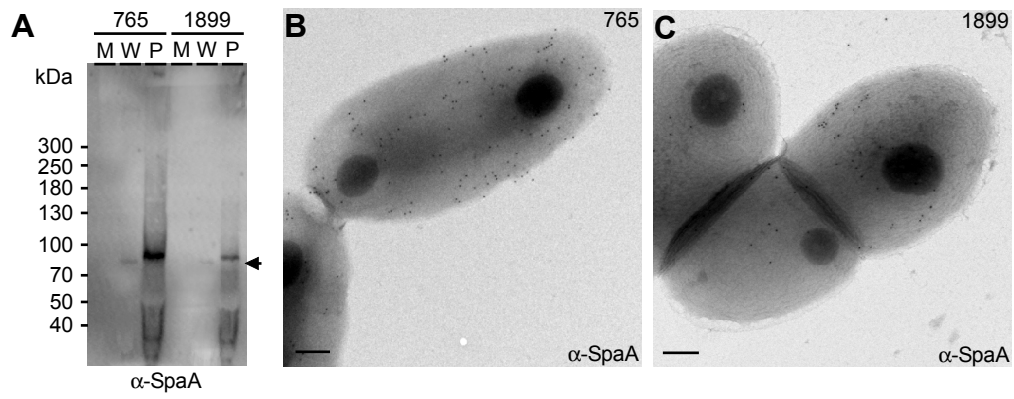


Figure S1: Broadway et al.

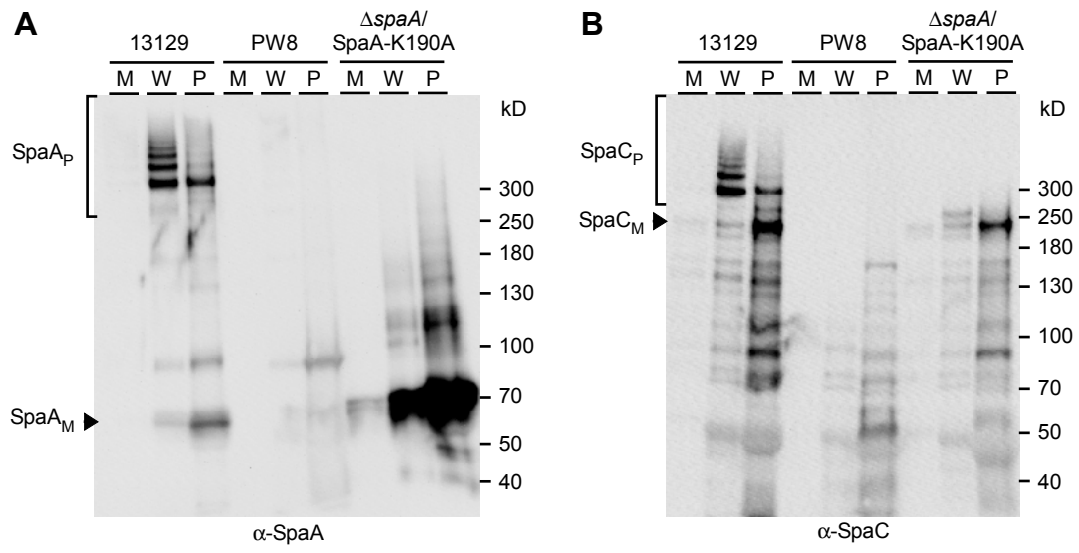


Figure S2: Broadway et al.

SUPPLEMENTAL MATERIALS

Figure Legends

Figure S1: Detection of SpaA in isolates 765 and 1899 by Western blotting and immuno-electron microscopy – (A) Culture medium (M), cell wall (W), and protoplast (P) fractions of isolates 765 (group 6) and 1899 (group 5) were subjected to immunoblotting with α -SpaA. Markers (kDa) and SpaA monomers (arrow) were indicated. (B-C) Surface display of SpaA in the two isolates was examined by immuno-electron microscopy with α -SpaA; bars of 200 nm.

Figure S2: Detection of SpaC in strain PW8 by Western blotting – Culture medium (M), cell wall (W), and protoplast (P) fractions of strains NCTC13128, PW8, and an isogenic *spaA* deletion mutant expressing SpaA-K190A ($\Delta spaA/pSpaA$ -K190A) were subjected to immunoblotting with α -SpaA (A) and α -SpaC (B). Strain $\Delta spaA/pSpaA$ -K190A was used as a control to detect SpaA and SpaC monomeric forms. Markers (kDa), Spa polymers (P), and monomers (M) were indicated.

Table S1: PCR^a and western blot (WB^b) analyses of corynebacterial strains

Group	Strain	Origin	Toxigenicity		SpaA		SpaD		SpaH		Reference
			Reported	PCR	PCR	WB	PCR	WB	PCR	WB	
1	C65	Canada	-	-	-	-	-	-	-	-	(1)
	C64	Canada	-	-	-	-	-	-	-	-	This study
	C78	Canada	-	-	-	-	-	-	-	-	(1)
	C50	Kazakhstan	-	-	-	-	-	-	-	-	(2)
	CD310	US, Ohio	ND ^c	-	-	-	-	-	-	-	This study
	CD317	US, Maine	ND	-	-	-	-	-	-	-	This study
	CD376	US, Colorado	ND	-	-	-	-	-	-	-	This study
	G4193	Russia	-	-	-	-	-	-	-	-	(3)
2	C52	Kazakhstan	-	-	+	+	-	-	-	-	(2)
	760	Russia	-	-	+	+	-	-	-	-	(3)
	CD318	US, Maine	ND	-	+	+	-	-	-	-	This study
3	PR26	US, South Dakota	-	-	+	+	-	-	+	+	(1)
	PR120	US, South Dakota	-	-	+	+	-	-	+	+	(1)
	CD364	US, New York	ND	-	+	+	-	-	+	+	This study
4	CD082	Russia	ND	+	-	-	-	-	-	-	This study
	496	Russia	+	+	-	-	-	-	-	-	(3)
	718	Russia	+	+	-	-	-	-	-	-	(3)
	713	Russia	+	+	-	-	-	-	-	-	(3)
	724	Russia	+	+	-	-	-	-	-	-	(3)
	C5276	US, South Dakota	+	+	-	-	-	-	-	-	(1)
	PR110	US, South Dakota	+	+	-	-	-	-	-	-	(1)
	G4219	US, South Dakota	+	+	-	-	-	-	-	-	(3)
5	1751	Ukraine	+	+	+	+	-	-	-	-	(3)
	G4212	Russia	+	+	+	+	-	-	-	-	(3)
	1899	Russia	+	+	+	+ ^(d)	-	-	-	-	(3)
	711	Russia	+	+	+	+	-	-	-	-	(3)
	G4182	Russia	+	+	+	+	-	-	-	-	(3)
	1709	Russia	+	+	+	+	-	-	-	-	(3)

	750	Russia	+	+	+	+	-	-	-	-	(3)
	PR75	US, South Dakota	+	+	+	+	-	-	-	-	(1)
	G4217	US, South Dakota	+	+	+	+	-	-	-	-	(1)
	A12	US, Virginia	+	+	+	+	-	-	-	-	(4)
	CD028	Canada	ND	+	+	+	-	-	-	-	This study
6	722	Russia	+	+	+	+	-	-	+	+	(3)
	E8277	US, South Dakota	+	+	+	+	+	+	-	-	(1)
	765	Russia	+	+	+	+(d)	+	+	-	-	(3)
7	1716	Russia	+	+	+	+	+	+	+	+	(3)
	1718	Russia	+	+	+	+	+	+	+	+	(3)
	1737	Russia	+	+	+	+	+	+	+	+	(3)
	1897	Ukraine	+	+	+	+	+	+	+	+	(3)
	G4174	Russia	+	+	+	+	+	+	+	+	(3)
	749	Russia	+	+	+	+	+	+	+	+	(3)
	13129	UK	+	+	+	+	+	+	+	+	(5)
	$\Delta spaABC$	Isogeny of 13129	ND	+	-	-	+	+	+	+	This study
	$\Delta spaABC/\Delta tox$	Isogeny of 13129	ND	-	-	-	+	+	+	+	This study
	$\Delta spaDEF$	Isogeny of 13129	ND	+	+	+	-	-	+	+	This study
	$\Delta spaHIG$	Isogeny of 13129	ND	+	+	+	+	+	-	-	This study
	$\Delta spaA-G$	Isogeny of 13129	ND	+	-	-	-	-	-	-	This study

^aSeveral pairs of primers were designed to PCR-amplify the coding sequence of *tox*, *spaA*, *spaD* and *spaH* (Table S2).

^bSeveral pairs of primers were designed to PCR-amplify the coding sequence of *tox*, *spaA*, *spaD* and *spaH*.

^cND – not determined.

^dOnly SpaA monomers were observed (see Fig. S1).

Table S2: Primers used in this study

Primers	Sequence 5' to 3'	Position ^(a)
spaA1_Foward	GTGGGGGAGAACGTGAAGTA	673-693
spaA1_Reverse	TCTTGGCCGGTAACAAACTC	1226-1246
spaA2_Foward	ACGTCACACTTGCCTCAGTG	62-81
spaA2_Reverse	CGGTGAAACCCTCGAACTTA	715-735
spaA3_Foward	CCGTACTIONCAGCGTGCAACTA	839-859
spaA3_Reverse	CCTGTGAGTGGCAGTTCAAA	1484-1504
spaB1_Foward	CACACGCACAAGAAGCAAAC	94-114
spaB1_Reverse	CGTCGAGTGCCTAGTAGTGC	628-648
spaB2_Foward	CTTCGCAGGAAGAGGACAAC	5-25
spaB2_Reverse	GATGAGGGTGCTGATGGTG	602-621
spaC1_Foward	AAGGCGTAGTGGATGAAACG	4312-4322
spaC1_Reverse	AAATCGGACGGTCTGTCAAC	4830-4850
spaC2_Foward	TGCAGACGGAAACGTGATAG	701-721
spaC2_Reverse	TTCGCACTGAGTTCCAACAG	1229-1249
spaD1_Foward	GGCATCTACCTCGTTGAGGA	193-213
spaD1_Reverse	TGCCGTCCTTCTTAACAACC	790-810
spaD2_Foward	TGCTGGTGAGCCACTAAATG	12-32
spaD2_Reverse	CCCGACTTGACCTTCTTCAG	614-634
spaD3_Foward	TACCCGAAGAACACCGAAAC	313-333
spaD3_Reverse	TTTTGACACCGTCCACAGTC	892-912
spaE1_Foward	GAAGGGCATCTACCTAGTCA	387-406
spaE1_Reverse	GTTGCGTCTTTCTCAT	658-677
spaE2_Foward	GATCAAAGCCGTGACCAA	336-354
spaE2_Reverse	AACCGTGGAGTTCGTCTT	546-564
spaF1_Foward	ATAAGAAGGTCAGCGTCAAA	232-252
spaF1_Reverse	ATCTACTGGGTCAGCTTCAA	797-817
spaF2_Foward	GCAACGGTTCTACTAACTGG	1477-1497
spaF2_Reverse	TGTGAGTACTGCTCAACTCG	2047-2067
spaH1_Foward	ACGGCAATATCGACTTCACC	140-160
spaH1_Reverse	TCAGACCCGACGTTAAGACC	674-694
spaH2_Foward	GAAGGTGGCTGAGGACAAAG	705-725
spaH2_Reverse	TACTCCGGACCACAAGTTCC	1265-1285
spaH3_Foward	GACCCGACCAAAGACGATAA	361-381
spaH3_Reverse	CAGCAGCATTCTTCAGCTTG	910-930
spaI1_Foward	GATAGAAGGCATCGATCTCA	203-223
spaI1_Reverse	TTCCTTACGGAACTTCTCTG	638-658
spaI2_Foward	GCTCAACATTTCCGATATTC	68-88
spaI2_Reverse	AAGATCGTCAGTGTCTTTGG	515-535
spaG1_Foward	ACCTACTATCTGCGTGAGGA	1961-1981
spaG1_Reverse	CGTCCTTCTTGGCTTTATTA	2483-2503
spaG2_Foward	AACGGTCTTGGTGTCTCTAA	371-391
spaG2_Reverse	GATGAGTCAAAGGCAAAGTC	924-944
Tox_Foward	AACGGCATTAGAGCATCCTG	717-737
Tox_Reverse	ATAGCTCTGCAACGCATCCT	1352-1372
16S_Foward	CGGAATTACTIONGGCGTAAAG	502-522
16S_Reverse	CTCTCATGAGTCCCCACCAT	1103-1123

^(a)Based on nucleotide sequences of indicated open-reading-frames of the sequenced strain NCTC13129.

Reference

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