

Figure S3 Alignment 834-bp insertion sequence and amino acid sequence of alpha-toxin variant

(a) Alignment of 834-bp insertion sequences in *plc* identified in strains S03 [16], CPBC16ML [17], and CP653 in the present study. Asterisk indicates identical nucleotide.

Ins-S03	GTGCGAGACGTTTCATAGTTGAAAAGACTATGCACATTCTAAATATAAGAGAATGGAGAAC	60
Ins-CP653	GTGCGAGACGTTTCATAGTTGAAAAGACTATGCACATTCTAAATATAAGAGAATGGAGAAC	60
Ins-CPBC16ML	GTGCGAGACGTTTCATAGTTGAAAAGACTATGCACATTCTAAATATAAGAGAATGGAGAAC	60

Ins-S03	TCTTAACCTGAAAAGTGAATGATAGGGTAACGCCTTGAAACGCTTTCTCTAATACTCCG	120
Ins-CP653	TCTTAACCTGAAAAGTGAATGATAGGGTAACGCCTTGAAACGCTTTCTCTAATACTCCG	120
Ins-CPBC16ML	TCTTAACCTGAAAAGTGAATGATAGGGTAACGCCTTGAAACGCTTTCTCTAATACTCCG	120

Ins-S03	ACATGCAGTAACCTATGTGTGAAAGTTATTGTGTGAAGCTCGGTGAAGTCGGCAGAAAATT	180
Ins-CP653	ACATGCAGTAACCTATGTGTGAAAGTTATTGTGTGAAGCTCGGTGAAGTCGGCAGAAAATT	180
Ins-CPBC16ML	ACATGCAGTAACCTATGTGTGAAAGTTATTGTGTGAAGCTCGGTGAAGTCGGCAGAAAATT	180

Ins-S03	ACCGTAATAGATAATATTATCTAACGAAAGTGTTCTAGAGGTAGAATATGTAGTTGATAT	240
Ins-CP653	ACCGTAATAGATAATATTATCTAACGAAAGTGTTCTAGAGGTAGAATATGTAGTTGATAT	240
Ins-CPBC16ML	ACCGTAATAGATAATATTATCTAACGAAAGTGTTCTAGAGGTAGAATATGTAGTTGATAT	240

Ins-S03	GCCGGGAGTCGTTAAAAACAGAATATGGTGAGAATGTTCCAATAGGAACTGACGAACTGG	300
Ins-CP653	GCCGGGAGTCGTTAAAAACAGAATATGGTGAGAATGTTCCAATAGGAACTGACGAACTGG	300
Ins-CPBC16ML	GCCGGGAGTCGTTAAAAACAGAATATGGTGAGAATGTTCCAATAGGAACTGACGAACTGG	300

Ins-S03	CGAAGGTACGCTCCTAAGCTATAAATATAGAAATATTAATAAATTGGTTTGAGGATTA	360
Ins-CP653	CGAAGGTACGCTCCTAAGCTATAAATATAGAAATATTAATAAATTGGTTTGAGGATTA	360
Ins-CPBC16ML	CGAAGGTACGCTCCTAAGCTATAAATATAGAAATATTAATAAATTGGTTTGAGGATTA	360

Ins-S03	GTAAGAATAGTACCCATGAAAATCCTTGATATGTTATAGGCATATAATAAGCCAATGGGG	420
Ins-CP653	GTAAGAATAGTACCCATGAAAATCCTTGATATGTTATAGGCATATAATAAGCCAATGGGG	420
Ins-CPBC16ML	GTAAGAATAGTACCCATGAAAATCCTTGATATGTTATAGGCATATAATAAGCCAATGGGG	420

Ins-S03	TAAAGTCAGCACCTAAGTTCTAGTATTGATAAAGTTAAGGGAACGTCGAAAGCTAAAGAC	480
Ins-CP653	TAAAGTCAGCACCTAAGTTCTAGTATTGATAAAGTTAAGGGAACGTCGAAAGCTAAAGAC	480
Ins-CPBC16ML	TAAAGTCAGCACCTAAGTTCTAGTATTGATAAAGTTAAGGGAACGTCGAAAGCTAAAGAC	480

Ins-S03	GTGGAGGATACACACCTATGAAGCGAAGAAAGGGAATACATAACCTTTCATAATCTTTAG	540
Ins-CP653	GTGGAGGATACACACCTATGAAGCGAAGAAAGGGAATACATAACCTTTCATAATCTTTAG	540
Ins-CPBC16ML	GTGGAGGATACACACCTATGAAGCGAAGAAAGGGAATACATAACCTTTCATAATCTTTAG	540

Ins-S03	TGAGAGTAGTGGCATGACTGATGAAGATTTTGTAAATGAAATTGGAGGAATAGCCACAAGT	600
Ins-CP653	TGAGAGTAGTGGCATGACTGATGAAGATTTTGTAAATGAAATTGGAGGAATAGCCACAAGT	600
Ins-CPBC16ML	TGAGAGTAGTGGCATGACTGATGAAGATTTTGTAAATGAAATTGGAGGAATAGCCACAAGT	600

Ins-S03	CAATTATTTATTTAAAAGAACTTAATCAATTCATGGATTCTAGTAAGATAAAAAAGGTCA	660
Ins-CP653	CAATTATTTATTTAAAAGAACTTAATCAATTCATGGATTCTAGTAAGATAAAAAAGGTCA	660
Ins-CPBC16ML	CAATTATTTATTTAAAAGAACTTAATCAATTCATGGATTCTAGTAAGATAAAAAAGGTCA	660

Ins-S03	CTGAATATAGAAAATTAGTGATAGACCATGGCAAGATATTTATATTAATACTTGAGATTA	720
Ins-CP653	CTGAATATAGAAAATTAGTGATAGACCATGGCAAGATATTTATATTAATACTTGAGATTA	720
Ins-CPBC16ML	CTGAATATAGAAAATTAGTGATAGACCATGGCAAGATATTTATATTAATACTTGAGATTA	720

Ins-S03	AGAAAAATGAATTTAATATATACTTGATGGAACGCCGTATGCGGTGAAAGTCGCACGTAC	780
Ins-CP653	AGAAAAATGAATTTAATATATACTTGATGGAACGCCGTATGCGGTGAAAGTCGCACGTAC	780
Ins-CPBC16ML	AGAAAAATGAATTTAATATATACTTGATGGAACGCCGTATGCGGTGAAAGTCGCACGTAC	780

Ins-S03	GGTGTGGAGCGGGGGAAAAATTGGAGATAACATCAAAGATTACCTATCGCTAT	834
Ins-CP653	GGTGTGGAGCGGGGGAAAAATTGGAGATAACATCAAAGATTACCTATCGCTAT	834
Ins-CPBC16ML	GGTGTGGAGCGGGGGAAAAATTGGAGATAACATCAAAGATTACCTATCGCTAT	834

(b) Alignment of deduced, spliced amino acid sequences of alpha-toxin in strains S03 and CP653. These sequences are translated through splicing from the original *plc* gene of strains S03 and CP653 (S03-spl, CP653-spl, respectively). Asterisk indicates identical amino acid. Different amino acids are shown in red.

	Signal peptide	
S03-spl	MKRKICKALICAALATSLWAGASTKVYAWDGKIDGTGTHAMIVTQGVSIENDLSKNEPE	60
CP653-spl	MKRKICKALICAALATSLWAGASTKVYAWDGKIDGTGTHAMIVTQGVSIENDLSKNEPE	60

S03-spl	SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPTDNNFSKDNSWYLAYSIPD	120
CP653-spl	SVRKNLEILKENMHELQLGSTYPDYDKNAYDLYQDHFWDPTDNNFSKDNSWYLAYSIPD	120

S03-spl	TGESQIRKFSALARYEWQRGNYKQATFYLGEMHYFGDIDTPYHPANVTAVDSAGHVKFE	180
CP653-spl	TGESQIRKFSALARYEWQRGNYKQATFYLGEMHYFGDIDTPYHPANVTAVDSAGHVKFE	180

S03-spl	TFAERKEQYKINTAGCKTNEAFYTDILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH	240
CP653-spl	TFAERKEQYKINTAGCKTNEAFYTDILKNKDFNAWSKEYARGFAKTGKSIYYSHASMSH	240

S03-spl	SWDWDYAAKVTLAN SQKGTAGYIYRFLHDVSEGN DPSVGKNVKELVAYISTSGEKDAGT	300
CP653-spl	SWDYWDYAAKVTLAN SQKGTAGYIYRFLHDVSEGN DPSVGKNVKELVAYISTSGEKDAGT	300
	*** *****	
S03-spl	DDYMYFGIKTKDGKTQEWEMDNP GDNFMTGSKD TYTFKLK DENLKIDDIQNMWIRKRKYT	360
CP653-spl	DDYMYFGIKTKDGKTQEWEMDNP GDNFMTGSKD TYTFKLK DENLKIDDIQNMWIRKRKYT	360

S03-spl	AFSDAYKPENIKIIANGKVVVDKDIN EWISGNSTYNIK	398
CP653-spl	ASDAYKPENIKIIANGKVVVDKDIN EWISGNSTYNIK	398
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