# Supplementary Materials: Characterization of the Deep-Sea *Streptomyces* sp. SCSIO 02999 Derived VapC/VapB Toxin-Antitoxin System in *Escherichia coli*

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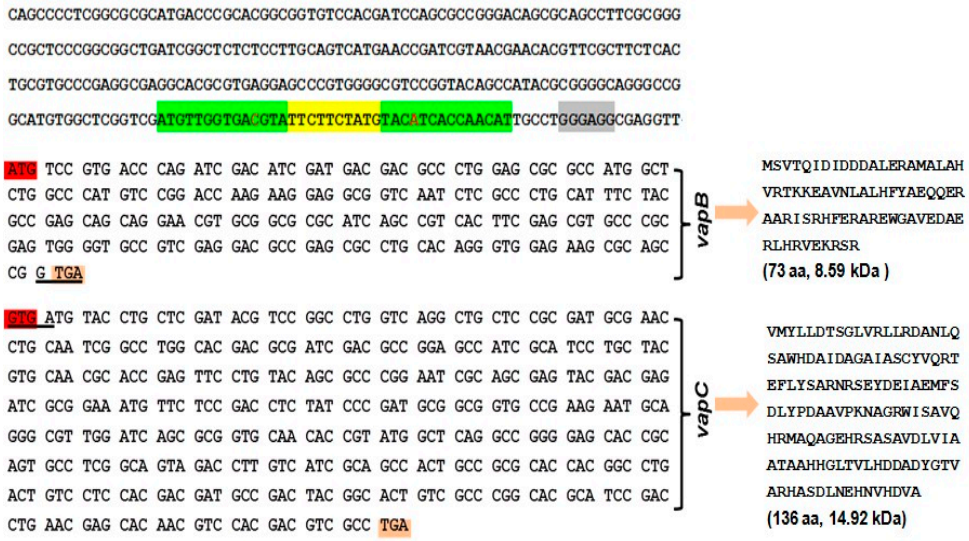


**Figure S1.** Gene and protein sequence of *vapBC* operon in *Streptomyces* sp. *SCSIO 02999*. The sequence encoding *vapB* and *vapC* are shown as indicated, and the protein sequences of VapB and VapC are also shown together with length and size. The 288 bp of *vapB* 5′ UTR (untranslated regions) is also shown; the palindrome is highlighted, the green indicates the palindrome (14 bp), the yellow indicates the gap, and the mismatch bases are shown in red letters. The ribosome binding site (RBS) is highlighted in gray. The start and stop codons for *vapB* and *vapC* are highlighted in red and orange, respectively. The overlapped four bases are underlined.

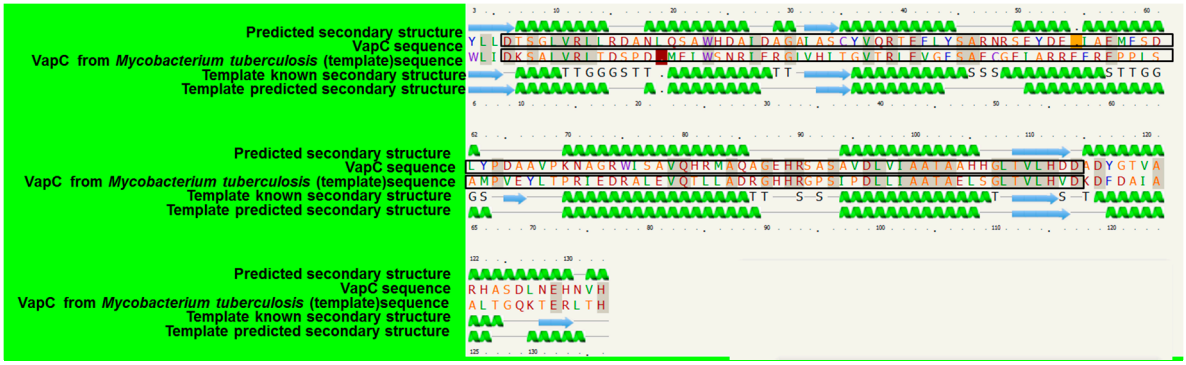


**Figure S2.** VapC in *Streptomyces* sp. SCSIO 02999 belongs to the PIN domain (PilT *N*-terminal) superfamily. The secondary structure of VapC was predicted using the online PHYRE2 (Protein Homology/analogY Recognition Engine V 2.0) server [1]. The $\alpha$-helices and β-sheets are shown. VapC was predicted to have a similar secondary structure with several VapC toxins of VapC/VapB TA family. Here, 130 residues (96% of VapC) have been modeled with 100.0% confidence by the known secondary structure of VapC from *Mycobacterium tuberculosis* with the single highest scoring template. The putative active sites (PIN domain) are boxed in open frames.

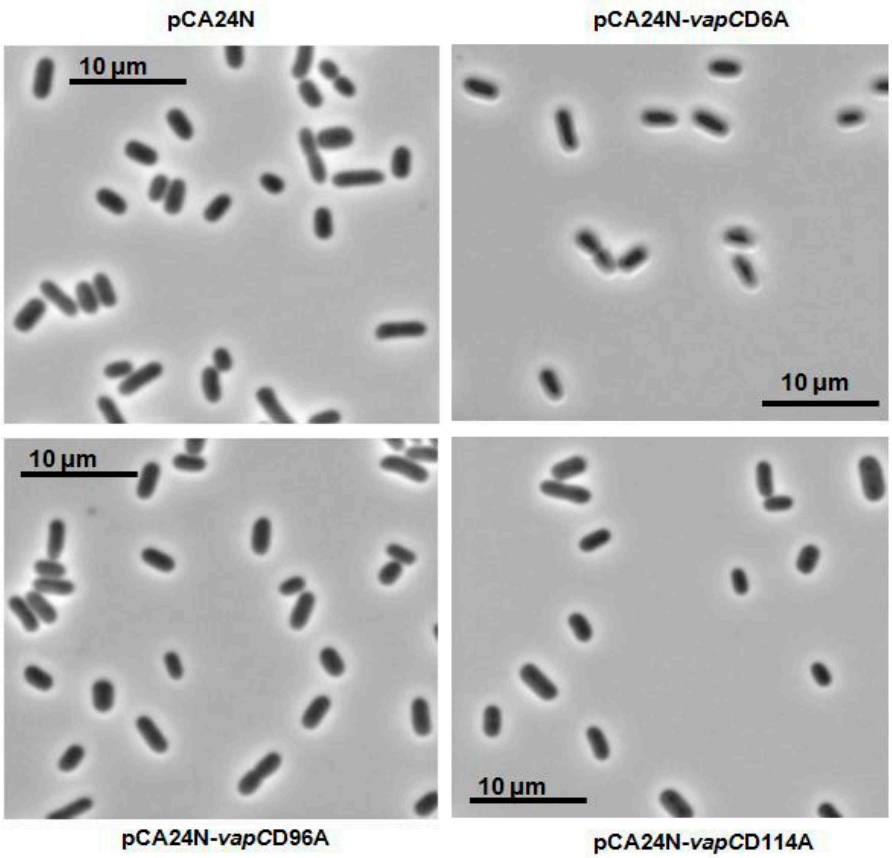


**Figure S3.** VapC mutant could not induce "bubble forming like" cells. Cells harboring pCA24N, pCA24N-*vap*CD6A, pCA24N-*vap*CD96A, and pCA24N-*vap*CD114A, respectively, were induced with 0.5 mM IPTG at $OD_{600}$ 1.0 for 5 h, and morphology of cells were observed under microscope.

**Table S1.** Oligonucleotides used for plasmid construction site-directed mutagenesis (target mutated nucleotides are in red font and highlighted yellow) and DNA sequencing. If an enzymatic restriction site is included in the sequence, the enzyme restriction site is underlined. f indicates forward primer and r indicates reverse primer, while P indicates promoter.

| Purpose/Name | Sequence (5′-3′) |
|---|---|
| **Plasmid construction** | |
| pCA24N-*vapB*-f | GCCTCCGTAACCCAGATCGACAT |
| pCA24N-*vapB*-r | CCACGGCTGCGCTTCTCCACCCT |
| pCA24N-*vapC*-f | GCCATGTACCTGCTCGATACGTC |
| pCA24N-*vapC*-r | CCGGCGACGTCGTGGACGTTGTG |
| pET28b-*vapB*-f | CTAGCCATGGGCATGTCCGTGACCCAGATCGACAT |
| pET28b-*vapB*-Chis-r | CCCAAGCTTCTAGTGGTGGTGGTGGTGGTGCCGGCTGCGCTTCTCCACCCTGTGCAGGC |
| pET28b-*vapC*-f | CTAGCCATGGGCATGTACCTGCTCGATACGTCCGG |
| pET28b-*vapC*-Chis-r | CCCAAGCTTCTAGTGGTGGTGGTGGTGGTGGGCGACGTCGTGGACGTTGTGCTCGTTCA |
| pET28b-*vapB-vapC*-r | CCCAAGCTTCTAGGCGACGTCGTGGACGTTGTGCTCGTTCA |
| pHGEI01-P*vapB-vapC-f* | CCGGAATTCGTCCACGATCCAGCGCCGGGACAG |
| pHGEI01-*vapB′*-r1 | AGCTGTTTCCTGTGTGAGATCTTTACAGAGCCATGGCGCGCTC |
| pHGEI01-*vapC′*-r1 | AGCTGTTTCCTGTGTGAGATCTTTAGGCGTCGATCGCGTCGTG |
| pHGEI01-*vapB-vapC*-r1 | AGCTGTTTCCTGTGTGAGATCTTCAGGCGACGTCGTGGACGTTGTG |
| pHGEI01-*vapB-vapC*-common-r2 | CGCGGATCCGTAATCATGGTCATAGCTGTTTCCTGTGTGAGATCT |
| **PCR and DNA sequencing** | |
| pCA24N-f | GATAACAATTTCACACAGAATT |
| pCA24N-r | GTCAGAGGTTTTCACCGTCATCA |
| pET28b-f | TAATACGACTCACTATAGGG |
| pET28b-r | TATGCTAGTTATTGCTCAG |
| **P*vapB-vapC*-f** | **TTCTCCAGCCCCTCGGCGCGCATGA** |
| P*vapB-vapC*-r | CATAACCTCGCCTCCCAGGCAATGTTGGTG |
| **QRT-PCR** | |
| *yoeB*-f | AGAACGCCATTTGAAGGTAAGG |
| *yoeB*-r | TGAGCAGTGAATCGTCGGTAAC |
| *yefM*-f | TGGAGAGGCTTGTGTTCTGATG |
| *yefM*-r | TTTCCGTTCCTTTGCCTGAT |
| *relE*-f | CACTAAAGGAATGGCGAAAGCT |

| | |
|---|---|
| *relE*-r | CCAACAGAAATCACGAAAACGA |
| *relB*-f | GGTAGCATTAACCTGCGTATTG |
| *relB*-r | AGCCGTTCTTTCACTATCTCCAC |
| *mazF*-f | TATGGGCGATCTGATTTGGG |
| *mazF*-r | TTTCTTCGTTGCTCCTCTTGC |
| *mqsR*-f | CACATACACGTTTGAGTCAGGTTAA |
| *mqsR*-r | ATCAGAGTAGGTGGTCATGCTTTT |
| *higB*-f | AACATAAAACGGAGTTGGTGGC |
| *higB*-r | ACGATGAACAGCGGTAAAGAAA |
| *hicA*-f | AATCTCAGGGCGTCGATGTAG |
| *hicA*-r | CGAGTTGTTTCAGGATTGCTTTA |
| *yafQ*-f | ATCAATAATACTTTACCGCTTCCA |
| *yafQ*-r | TTATCGGTAAGTTTGTAAATCAGGA |
| *yafO*-f | TTTCCTATAAGCGTGACGGTGTT |
| *yafO*-r | GAGGTTCAGGTTTCAGAATGGC |
| *yhaV*-f | ATCACGGTCAATCCATCATCAC |
| *yhaV*-r | GCTGAATACGGTATAGGCATCTGT |
| ***chpB*-f** | **GTTCAAGCCTTTAATCAACTGGG** |
| *chpB*-r | TAATAACGCCTCTTCCACCACC |
| *rbn*-f | AGTCGCGGCCATAGCTCTAC |
| *rbn*-r | GAAATCATTCGCCAGTTCAGTC |
| *ralR*-f | CATCAGTAACGGTGAAAGCCA |
| *ralR*-r | CCAGTGGTTCGTTTATTCCA |
| *ghoT-f* | CCTTTGTCATTATCTGGTTTATCTCAC |
| *ghoT-r* | AAAGAGAGAAAAAAGTAATGCCACAG |
| *purA*-f | GGGCCTGCTTATGAAGATAAAGT |
| *purA*-r | TCAACCACCATAGAAGTCAGGAT |
| *rrsG-f* | TATTGCACAATGGGCGCAAG |
| *rrsG-r* | ACTTAACAAACCGCCTGCGT |
| **Single Amino Acid Substitution** | |
| *vap*CD6A-f | CATGTACCTGCTCGCTACGTCCGGCCTGG |
| *vap*CD6A -r | CCAGGCCGGACGTAGCGAGCAGGTACATG |
| *vap*CD96A -f | TGCCTCGGCAGTAGCCCTTGTCATCGCAG |
| *vap*CD96A -r | CTGCGATGACAAGGGCTACTGCCGAGGCA |

| | |
|---|---|
| *vap*CD114A -f | GTCCTCCACGACGCTGCCGACTACGGC |
| *vap*CD114A -r | GCCGTAGTCGGCAGCGTCGTGGAGGAC |

## Reference

1. Kelley, L.A.; Sternberg, M.J. Protein structure prediction on the web: A case study using the phyre server. *Nat. Protoc.* **2009**, *4*, 363–371.