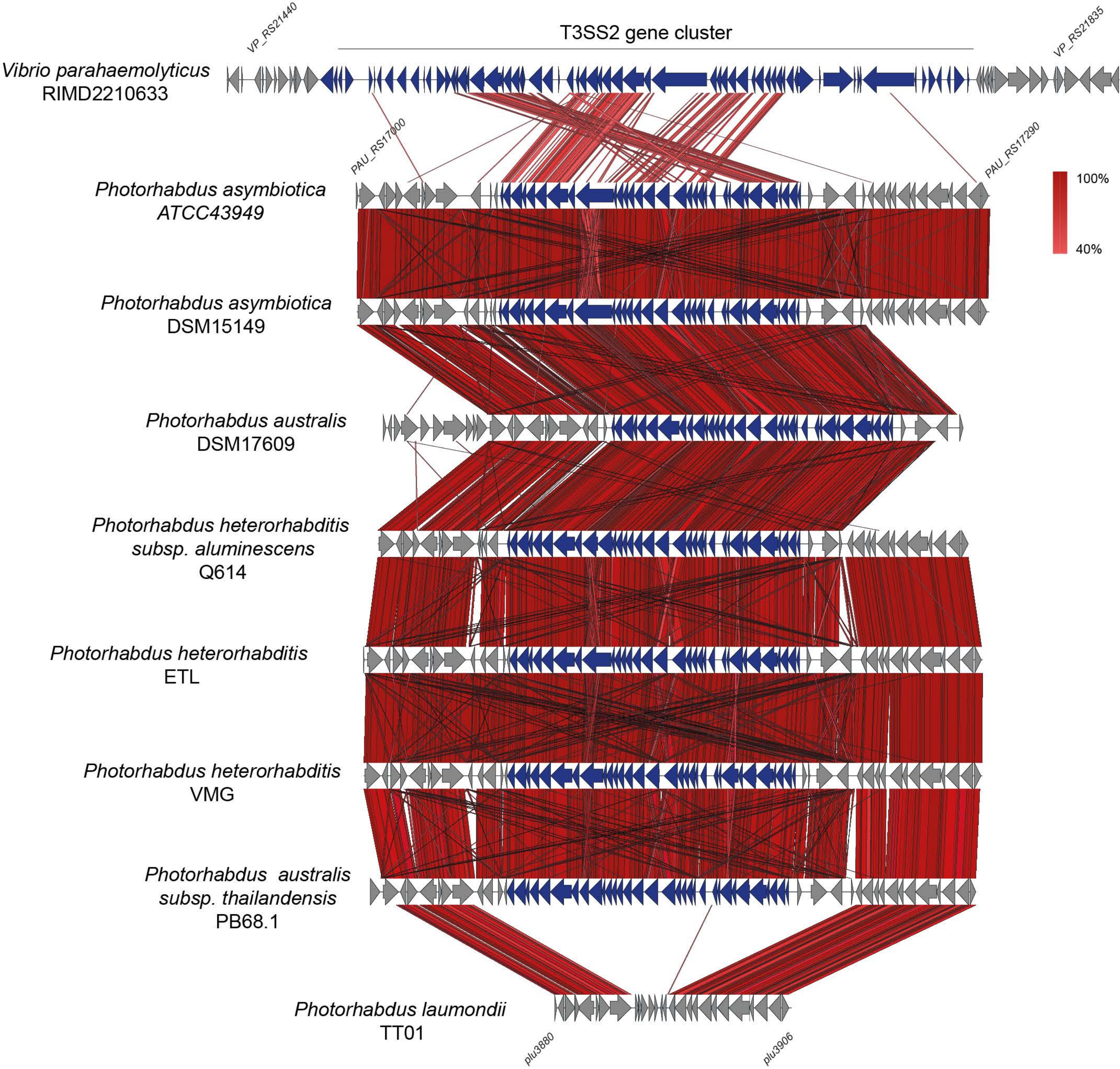


Fig. S1. Gene synteny between the T3SS2 gene clusters of *V. parahaemolyticus* RIMD2210633 and the identified T3SS2 gene clusters of Photorhabdus species. tBLASTx alignments were performed and visualized using Easyfig. Genes encoding T3SS-related genes are highlighted in blue.

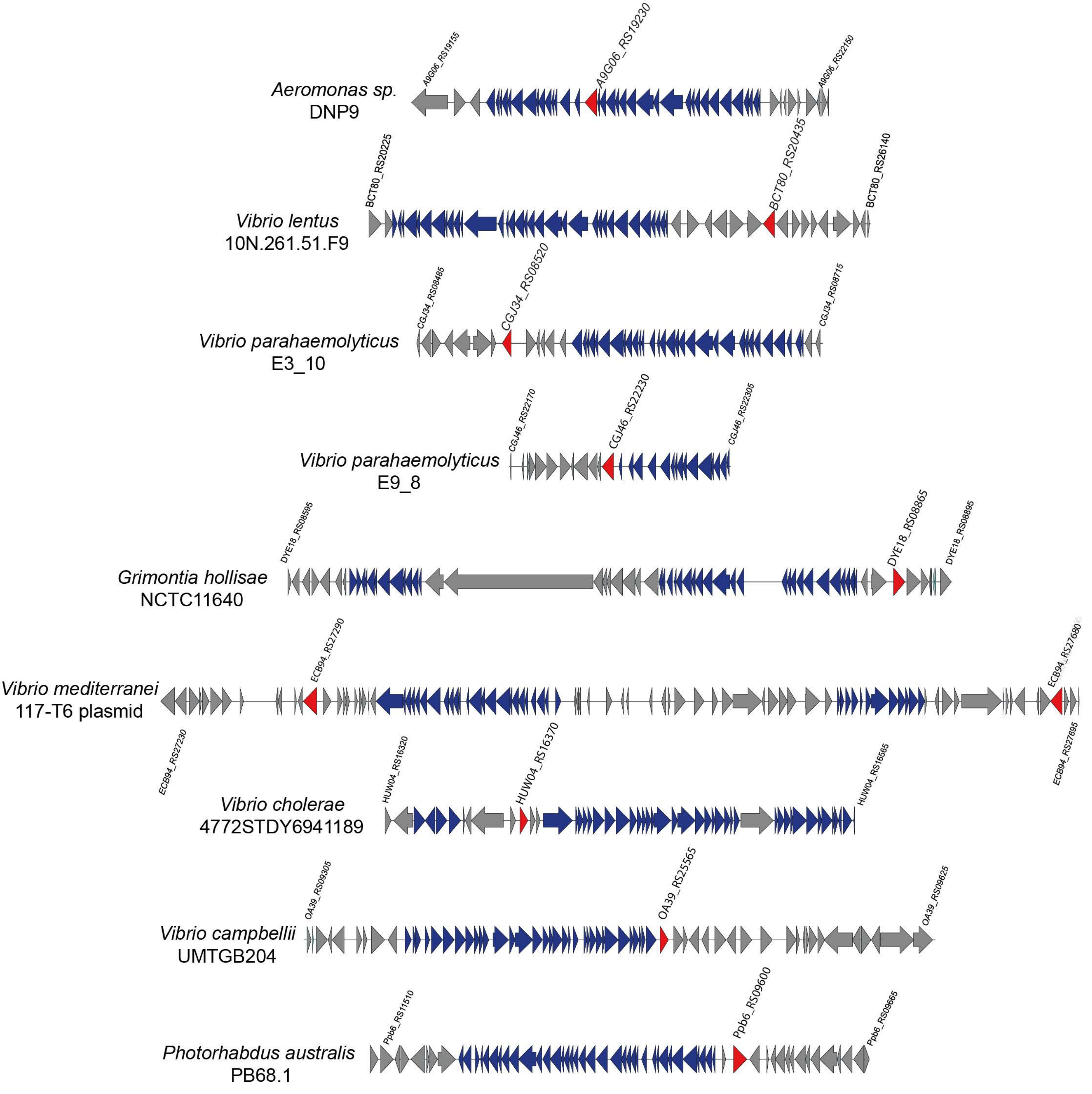
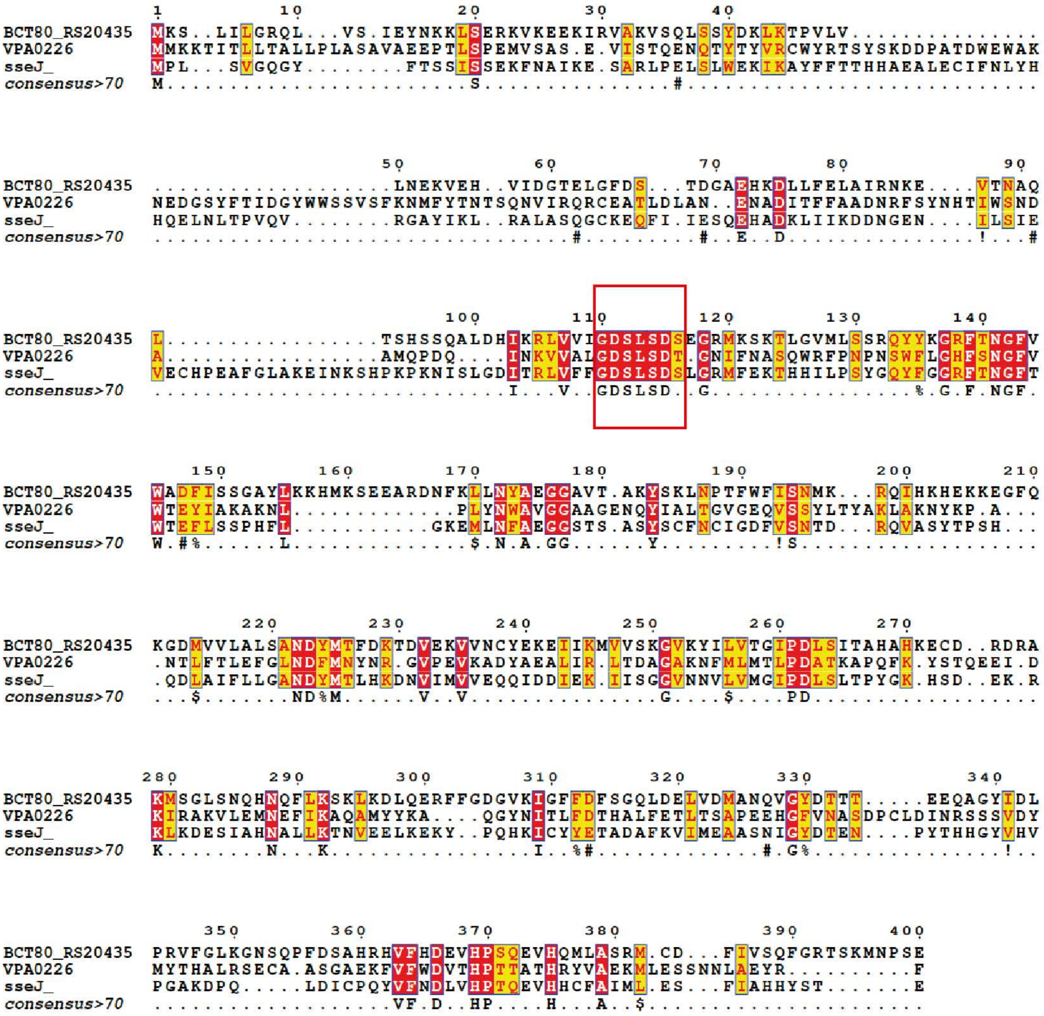
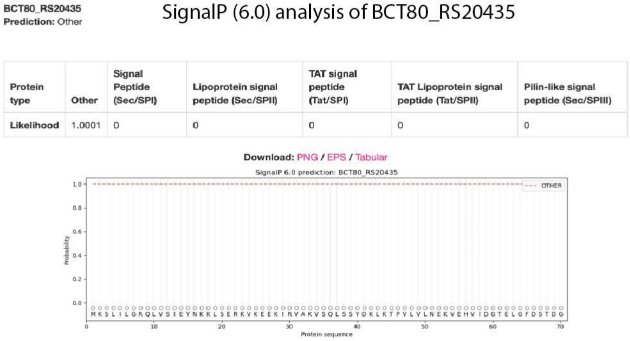


Fig. S2. Genetic context of novel effector candidates in representative T3SS2 gene clusters. The T3SS2 gene clusters are shown in blue, and each novel effector candidate is highlighted in red.



```
                1         10            20        30        40
BCT80_RS20435   MKS..LILGRQL...VS.IEYNKKLSERKVKEEKIRVAKVSQLSSYDKLKTPVLV...............
VPA0226         MMKKTITLLTALLPLASAVAEEPTLSPEMVSAS.E.VISTQENQTYTYVRCWYRTSYSKDDPATDWEWAK
sseJ_           MPL...SVGQGY........FTSSISSEKFNAIKE.SARLPELSLWEKIKAYFFTTHHAEALECIFNLYH
consensus>70    M........................S.................#..........................

                                  50          60           70        80            90
BCT80_RS20435   ...................LNEKVEH..VIDGTELGFDS...TDGAEHKDLLFELAIRNKE....VTNAQ
VPA0226         NEDGSYFTIDGYWWSSVSFKNMFYTNTSQNVIRQRCEATLDLAN..ENADITFFAADNRFSYNHTIWSND
sseJ_           HQELNLTPVQV........RGAYIKL..RALASQGCKEQFI.IESQEHADKLIIKDDNGEN....ILSIE
consensus>70    .................................#.........#..E..D...............!...#

                                       100       110       120       130       140
BCT80_RS20435   L.................TSHSSQALDHIKRLVVIGDSLSDSEGRMKSKTLGVMLSSRQYYKGRFTNGFV
VPA0226         A.................AMQPDQ....INKVVALGDSLSDT.GNIFNASQWRFPNPNSWFLGHFSNGFV
sseJ_           VECHPEAFGLAKEINKSHPKPKNISLGDITRLVFFGDSLSDSLGRMFEKTHHILPSYGQYFGGRFTNGFT
consensus>70    ............................I...V..GDSLSD..G................%.G.F.NGF.

                   150       160       170       180        190          200       210
BCT80_RS20435   WADFISSGAYLKKHMKSEEARDNFKLLNYAEGGAVT.AKYSKLNPTFWFISNMK...RQIHKHEKKEGFQ
VPA0226         WTEYIAKAKNL.............PLYNWAVGGAAGENQYIALTGVGEQVSSYLTYAKLAKNYKP.A...
sseJ_           WTEFLSSPHFL...........GKEMLNFAEGGSTS.ASYSCFNCIGDFVSNTD...RQVASYTPSH...
consensus>70    W.#%......L..............$.N.A.GG......Y.........!S...................

                       220       230       240       250       260       270
BCT80_RS20435   KGDMVVLALSANDYMTFDKTDVEKVVNCYEKEIIKMVVSKGVKYILVTGIPDLSITAHAHKECD..RDRA
VPA0226         .NTLFTLEFGLNDFMNYNR.GVPEVKADYAEALIR.LTDAGAKNFMLMTLPDATKAPQFK.YSTQEEI.D
sseJ_           .QDLAIFLLGANDYMTLHKDNVIMVVEQQIDDIEK.IISGGVNNVLVMGIPDLSLTPYGK.HSD..EK.R
consensus>70    ...$.......ND%M......V..V...............G....$....PD..................

               280       290       300       310       320       330            340
BCT80_RS20435   KMSGLSNQHNQFLKSKLKDLQERFFGDGVKIGFFDFSGQLDELVDMANQVGYDTTT.....EEQAGYIDL
VPA0226         KIRAKVLEMNEFIKAQAMYYKA....QGYNITLFDTHALFETLTSAPEEHGFVNASDPCLDINRSSSVDY
sseJ_           KLKDESIAHNALLKTNVEELKEKY..PQHKICYYETADAFKVIMEAASNIGYDTEN....PYTHHGYVHV
consensus>70    K........N...K................I..%#.............#.G%...............!..

                    350       360       370       380           390       400
BCT80_RS20435   PRVFGLKGNSQPFDSAHRHVFHDEVHPSQEVHQMLASRM.CD...FIVSQFGRTSKMNPSE
VPA0226         MYTHALRSECA.ASGAEKFVFWDVTHPTTATHRYVAEKMLESSNNLAEYR..........F
sseJ_           PGAKDPQ.....LDICPQYVFNDLVHPTQEVHHCFAIML.ES...FIAHHYST.......E
consensus>70    ...................VF.D..HP....H...A..$......................
```

SignalP (6.0) analysis of BCT80_RS20435



BCT80_RS20435
Prediction: Other

Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPI)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
Likelihood	1.0001	0	0	0	0	0

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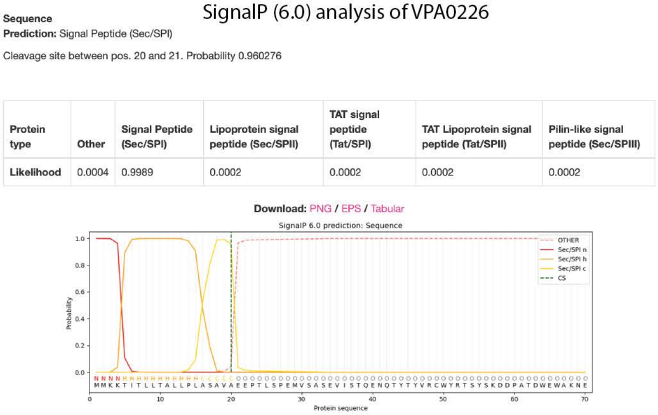
SignalP (6.0) analysis of VPA0226



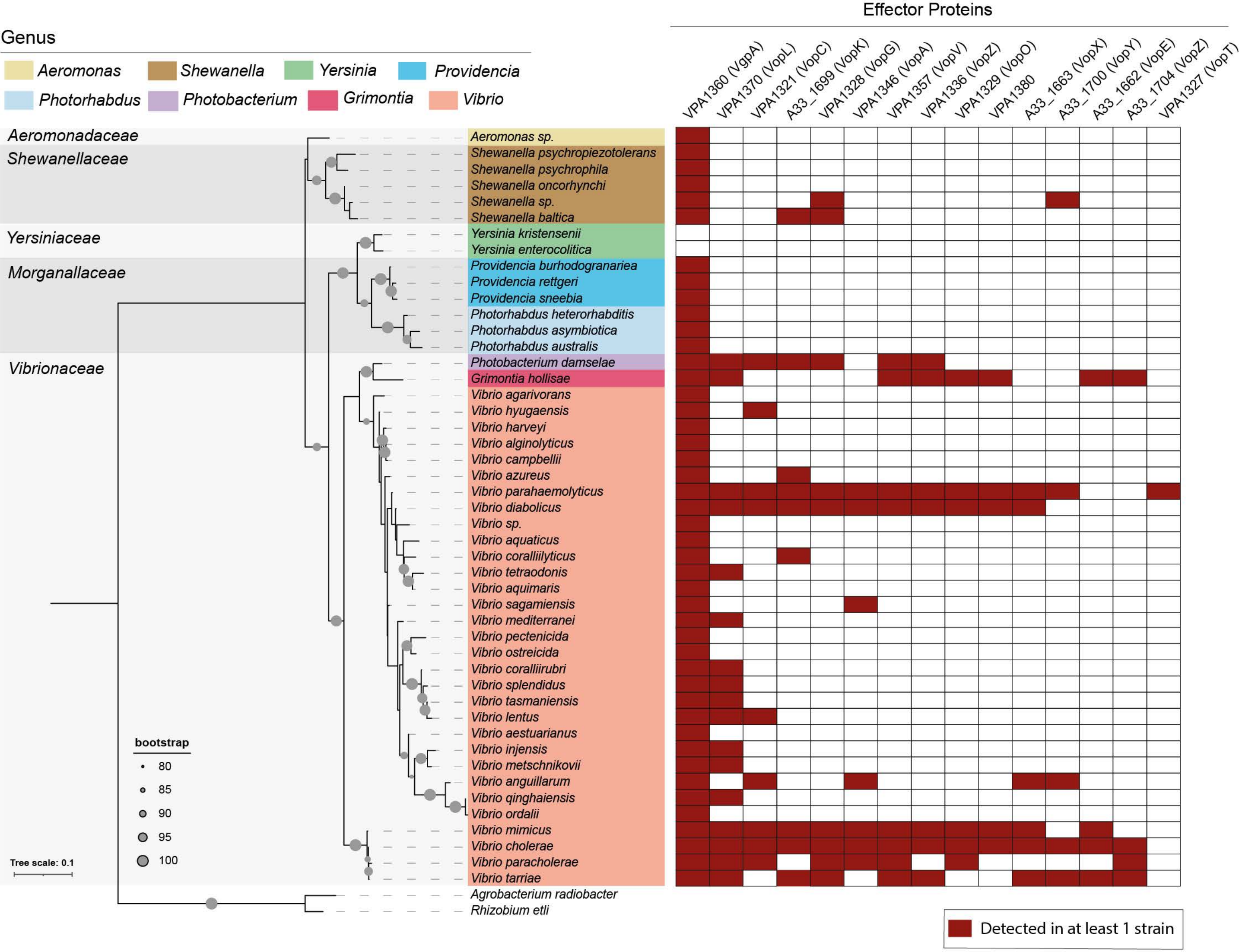
Sequence
Prediction: Signal Peptide (Sec/SPI)

Cleavage site between pos. 20 and 21. Probability 0.960276

Protein type	Other	Signal Peptide (Sec/SPI)	Lipoprotein signal peptide (Sec/SPII)	TAT signal peptide (Tat/SPI)	TAT Lipoprotein signal peptide (Tat/SPII)	Pilin-like signal peptide (Sec/SPIII)
Likelihood	0.0004	0.9989	0.0002	0.0002	0.0002	0.0002

Download: PNG / EPS / Tabular

Fig. S3. Multiple sequence alignment of the amino acid sequence of the predicted effector BCT80_RS20435 and the secreted lipase VPA0226 and the Salmonella T3SS effector protein SseJ. The alignment was performed using T-Coffee Expresso and visualized by ESPript 3.0. Amino acids within a red background correspond to positions with 100% identity; amino acids with a yellow background correspond to positions with >70% identity SignalP analysis shows that BCT80_RS20435, unlike VPA0226, does not harbor Sec or TAT secretion signals.



	VPA1360 (VgpA)	VPA1370 (VopL)	VPA1321 (VopC)	A33_1699 (VopK)	VPA1328 (VopG)	VPA1346 (VopA)	VPA1357 (VopV)	VPA1336 (VopZ)	VPA1329 (VopO)	VPA1380	A33_1663 (VopX)	A33_1700 (VopY)	A33_1662 (VopE)	A33_1704 (VopZ)	VPA1327 (VopT)
Aeromonas sp.	■														
Shewanella psychropiezotolerans	■														
Shewanella psychrophila	■														
Shewanella oncorhynchi	■														
Shewanella sp.	■				■							■			
Shewanella baltica	■			■	■										
Yersinia kristensenii															
Yersinia enterocolitica															
Providencia burhodogranariea	■														
Providencia rettgeri	■														
Providencia sneebia	■														
Photorhabdus heterorhabditis	■														
Photorhabdus asymbiotica	■														
Photorhabdus australis	■														
Photobacterium damselae	■	■	■	■	■		■	■							
Grimontia hollisae	■	■					■	■	■	■			■	■	
Vibrio agarivorans	■														
Vibrio hyugaensis	■		■												
Vibrio harveyi	■														
Vibrio alginolyticus	■														
Vibrio campbellii	■														
Vibrio azureus	■			■											
Vibrio parahaemolyticus	■	■	■	■	■	■	■	■	■	■	■	■			■
Vibrio diabolicus	■	■	■	■	■	■	■	■	■	■	■				
Vibrio sp.	■														
Vibrio aquaticus	■														
Vibrio coralliilyticus	■			■											
Vibrio tetraodonis	■	■													
Vibrio aquimaris	■														
Vibrio sagamiensis	■					■									
Vibrio mediterranei	■	■													
Vibrio pectenicida	■														
Vibrio ostreicida	■														
Vibrio coralliirubri	■	■													
Vibrio splendidus	■	■													
Vibrio tasmaniensis	■	■													
Vibrio lentus	■	■	■												
Vibrio aestuarianus	■														
Vibrio injensis	■	■													
Vibrio metschnikovii	■	■													
Vibrio anguillarum	■		■			■					■	■			
Vibrio qinghaiensis	■	■													
Vibrio ordalii	■														
Vibrio mimicus	■	■	■	■	■	■	■	■	■	■	■		■		
Vibrio cholerae	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
Vibrio paracholerae	■	■	■		■	■	■		■					■	
Vibrio tarriae	■	■		■	■		■	■			■	■	■	■	

■ Detected in at least 1 strain

Fig. S4. Distribution of T3SS2 effector proteins among bacterial species. The distribution matrix shows the detection of each effector protein in at least one bacterial strain in red. The phylogenetic tree is the same tree as Fig 2.