**Supplementary file 1a.** Strains and plasmids used in this study.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Strain** | **Genotype** | **Description** | | **Source** |
| *E. coli* |  |  |  | |
| BL21(DE3) | *F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacI lacUV5T7p07 ind1 sam7 nin5]) [malB+]K-12(λS)* | Strain used for protein expression | Lab stock | |
| DH5α | *F- Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 phoA supE44 thi-1 gyrA96 relA1 λ-* | Strain used for cloning | Invitrogen | |
| T-fast | *F' proA+ B+ lacIq ∆lacZM15 / fhuA2 ∆(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)TetR endA1 thi-1 ∆(hsdS-mcrB)5* | Strain used for cloning | TIANGEN | |
| MG1655 | *F-*, *lambda-, rph-1* | K-12 wild-type strain used as prey for T6SS killing | Lab stock | |
| *Aeromonas dhakensis* SSU | WT | Parental strain | [1] | |
|  | Δ*vasK* | T6SS null, in-frame deletion of *vasK* | [1] | |
|  | Δ*tseP* | In-frame deletion of *tseP* | [1] | |
|  | Δ*3eff*, *vipA*-*sfgfp* | In-frame deletion of *tseP*, *tseI*, and *tseC*, and fusion expression of sfGFP protein and VipA protein. | This study | |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Plasmid** | **Description** | **Source** |
| pBAD24 | pBAD24Kan | Arabinose inducible expression plasmid, kanamycin resistance | Lab stock |
|  | pBAD24kan-VgrG2-TseP-V5 | Arabinose inducible expression of VgrG2 and TseP | This study |
|  | pBAD24kan-VgrG2-TsePN-V5 | Arabinose inducible expression of VgrG2 and TsePN | This study |
|  | pBAD24kan-VgrG2-TsePC-V5 | Arabinose inducible expression of VgrG2 and TsePC | This study |
|  | pBAD24kan-VgrG2-TsePH19A-V5 | Arabinose inducible expression of VgrG2 and TseP H19A | This study |
|  | pBAD24kan-VgrG2-TsePH23A-V5 | Arabinose inducible expression of VgrG2 and TsePH23A | This study |
|  | pBAD24kan-VgrG2-TsePH109A-V5 | Arabinose inducible expression of VgrG2 and TsePH109A | This study |
|  | pBAD24kan-VgrG2-TsePH339A-V5 | Arabinose inducible expression of VgrG2 and TsePH339A | This study |
|  | pBAD24kan-VgrG2-TsePH359A-V5 | Arabinose inducible expression of VgrG2 and TsePH359A | This study |
|  | pBAD24kan-VgrG2-TsePE539A-V5 | Arabinose inducible expression of VgrG2 and TsePE539A | This study |
|  | pBAD24kan-VgrG2-TsePEFhandmut-V5 | Arabinose inducible expression of VgrG2 and TsePEFhandmut | This study |
| pBAD18 | pBAD18Cm | Arabinose inducible expression plasmid, choloramphenicol resistance | Lab stock |
| pET28a |  | IPTG inducible expression plasmid, gentamycin resistant | Lab stock |
|  | pET28a-His-SUMO-TseP | IPTG inducible expression of SUMO-TseP fusion with an N-terminal 6×His tag | Lab stock |
|  | pET28a-His-SUMO-TsePE663A | IPTG inducible expression of SUMO-TsePE663A fusion with an N-terminal 6×His tag | [2] |
|  | pET28a-His-SUMO-TsePN | IPTG inducible expression of SUMO-TseP fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-H19A | IPTG inducible expression of SUMO-TsePN-H19A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-H23A | IPTG inducible expression of SUMO-TsePN-H23A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-H109A | IPTG inducible expression of SUMO-TsePN-H109A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-H339A | IPTG inducible expression of SUMO-TsePN-H339A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-H359A | IPTG inducible expression of SUMO-TsePN-H359A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePN-E539A | IPTG inducible expression of SUMO-TsePN-E539A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC | IPTG inducible expression of SUMO-TsePC fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC-E663D | IPTG inducible expression of SUMO-TsePC-E663D fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC4+ | IPTG inducible expression of SUMO-TsePC4+ fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC-D604K | IPTG inducible expression of SUMO-TsePC-D604K fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC-D841K | IPTG inducible expression of SUMO-TsePC-D841K fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsePC-D841K/E845K | IPTG inducible expression of SUMO-TsePC-D841K/E845K fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-TsiP | IPTG inducible expression of SUMO-TsiP fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-VgrG2 | IPTG inducible expression of SUMO-VgrG2 fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-AHA\_1849 | IPTG inducible expression of SUMO-AHA\_1849 fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-AHA\_1849N | IPTG inducible expression of SUMO-AHA\_1849N fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-AHA\_1849N-H369A | IPTG inducible expression of SUMO- AHA\_1849N-H369A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-AHA\_1849C | IPTG inducible expression of SUMO-AHA\_1849C fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-AHA\_1849C-D689A | IPTG inducible expression of SUMO-AHA\_1849C-D689A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204 | IPTG inducible expression of SUMO-PSPTO\_5204 fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204N | IPTG inducible expression of SUMO-PSPTO\_5204N fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204N-H106A | IPTG inducible expression of SUMO-PSPTO\_5204N-H106A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204N-H271A | IPTG inducible expression of SUMO-PSPTO\_5204N-H271A fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204C | IPTG inducible expression of SUMO-PSPTO\_5204C fusion with an N-terminal 6×His tag | This study |
|  | pET28a-His-SUMO-PSPTO\_5204C-E397A | IPTG inducible expression of SUMO-PSPTO\_5204C-E397A fusion with an N-terminal 6×His tag | This study |

**Supplementary file 1b.** The sequence identity of the N-terminal of TseP and homologs. Sequence identity was calculated using ClustalW web server. Colors are assigned from blue to red indicating low to high sequence similarity, respectively.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| **TseP** |  | 41.8 | 88.2 | 88.7 | 47.1 | 12.4 | 14.3 | 12.1 | 13 | 12.1 | 13.3 | 12.6 | 12.3 | 12.5 | 16.7 |
| NCTC12917\_01703 | 41.8 |  | 42.9 | 41.1 | 60.6 | 14.9 | 13.7 | 13 | 14.7 | 13.9 | 13.3 | 10.9 | 10 | 14.4 | 17.4 |
| WP3S18E02\_26330 | 88.2 | 42.9 |  | 88.5 | 46.8 | 13 | 12.8 | 10.9 | 15.1 | 12 | 12.8 | 12.4 | 12 | 13.7 | 17 |
| AHA\_1849 | 88.7 | 41.1 | 88.5 |  | 47.4 | 12.5 | 11.6 | 12.6 | 13.3 | 11.7 | 13 | 13.1 | 15.7 | 13.1 | 17.1 |
| PLESHI\_03291 | 47.1 | 60.6 | 46.8 | 47.4 |  | 14.5 | 13.9 | 13.4 | 16.3 | 12.6 | 13.5 | 11.1 | 14.6 | 13.8 | 17.5 |
| CY652\_03435 | 12.4 | 14.9 | 13 | 12.5 | 14.5 |  | 15.6 | 12.8 | 13.5 | 42.1 | 13.2 | 17.4 | 13.5 | 12.2 | 14.9 |
| BTO02\_30765 | 14.3 | 13.7 | 12.8 | 11.6 | 13.9 | 15.6 |  | 14 | 13.7 | 13.3 | 18.4 | 13.3 | 13.1 | 34.9 | 12.8 |
| PSPTO\_5204 | 12.1 | 13 | 10.9 | 12.6 | 13.4 | 12.8 | 14 |  | 12.3 | 10.7 | 13.2 | 13.2 | 15.3 | 13.4 | 14.6 |
| Dda3937\_03232 | 13 | 14.7 | 15.1 | 13.3 | 16.3 | 13.5 | 13.7 | 12.3 |  | 13.3 | 17.2 | 14.2 | 14.4 | 17.4 | 18.1 |
| EBL\_c11670 | 12.1 | 13.9 | 12 | 11.7 | 12.6 | 42.1 | 13.3 | 10.7 | 13.3 |  | 13.7 | 12.3 | 10.7 | 11.6 | 12.8 |
| SAMN04488136\_12163 | 13.3 | 13.3 | 12.8 | 13 | 13.5 | 13.2 | 18.4 | 13.2 | 17.2 | 13.7 |  | 11.8 | 13.5 | 17.3 | 14.9 |
| ABW06\_23985 | 12.6 | 10.9 | 12.4 | 13.1 | 11.1 | 17.4 | 13.3 | 13.2 | 14.2 | 12.3 | 11.8 |  | 11.6 | 12.4 | 12.2 |
| SAMN05216206\_1427 | 12.3 | 10 | 12 | 15.7 | 14.6 | 13.5 | 13.1 | 15.3 | 14.4 | 10.7 | 13.5 | 11.6 |  | 15.9 | 16.3 |
| C4K68\_12090Tola\_1753 | 12.5 | 14.4 | 13.7 | 13.1 | 13.8 | 12.2 | 34.9 | 13.4 | 17.4 | 11.6 | 17.3 | 12.4 | 15.9 |  | 13.8 |
| Tola\_1753 | 16.7 | 17.4 | 17 | 17.1 | 17.5 | 14.9 | 12.8 | 14.6 | 18.1 | 12.8 | 14.9 | 12.2 | 16.3 | 13.8 |  |

**Supplementary file 1c.** The sequence identity of the C-terminal of TseP and homologs. Sequence identity was calculated using ClustalW web server. Colors are assigned from blue to red indicating low to high sequence similarity, respectively.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| **TseP** |  | 10 | 11.8 | 12.4 | 13.6 | 48.1 | 48.9 | 49.8 | 20.7 | 24.2 | 19.8 | 17.4 | 10.1 | 19.3 | 12.1 |
| NCTC12917\_01703 | 10 |  | 69.2 | 18.7 | 11.4 | 9.5 | 11.4 | 11.4 | 11.8 | 9.5 | 11.4 | 11.4 | 14.7 | 12.3 | 17.1 |
| WP3S18E02\_26330 | 11.8 | 69.2 |  | 17.1 | 11.3 | 12.2 | 10.9 | 11.8 | 11.3 | 10.9 | 11.8 | 10 | 14.9 | 11.8 | 15.4 |
| AHA\_1849 | 12.4 | 18.7 | 17.1 |  | 12.4 | 10.9 | 10.4 | 14 | 11.4 | 11.4 | 10.9 | 12.4 | 15 | 12.4 | 13.5 |
| PLESHI\_03291 | 13.6 | 11.4 | 11.3 | 12.4 |  | 14.2 | 14 | 14.1 | 13.9 | 13.1 | 13.2 | 13 | 13.6 | 14.6 | 11.6 |
| CY652\_03435 | 48.1 | 9.5 | 12.2 | 10.9 | 14.2 |  | 46.4 | 49.8 | 21 | 23.6 | 19.8 | 20.6 | 11.4 | 21.5 | 11.6 |
| BTO02\_30765 | 48.9 | 11.4 | 10.9 | 10.4 | 14 | 46.4 |  | 50.6 | 21.3 | 22.6 | 20.7 | 20.4 | 10.1 | 22.7 | 9.8 |
| PSPTO\_5204 | 49.8 | 11.4 | 11.8 | 14 | 14.1 | 49.8 | 50.6 |  | 22.4 | 24.6 | 20.3 | 17 | 10.5 | 21 | 10.3 |
| Dda3937\_03232 | 20.7 | 11.8 | 11.3 | 11.4 | 13.9 | 21 | 21.3 | 22.4 |  | 58.5 | 26.4 | 21.3 | 10.1 | 33.9 | 9.4 |
| EBL\_c11670 | 24.2 | 9.5 | 10.9 | 11.4 | 13.1 | 23.6 | 22.6 | 24.6 | 58.5 |  | 25.1 | 23.3 | 10.1 | 35.2 | 8.5 |
| SAMN04488136\_12163 | 19.8 | 11.4 | 11.8 | 10.9 | 13.2 | 19.8 | 20.7 | 20.3 | 26.4 | 25.1 |  | 21.1 | 9.7 | 25.1 | 10.3 |
| ABW06\_23985 | 17.4 | 11.4 | 10 | 12.4 | 13 | 20.6 | 20.4 | 17 | 21.3 | 23.3 | 21.1 |  | 12.7 | 18.9 | 10.7 |
| SAMN05216206\_1427 | 10.1 | 14.7 | 14.9 | 15 | 13.6 | 11.4 | 10.1 | 10.5 | 10.1 | 10.1 | 9.7 | 12.7 |  | 11 | 50.4 |
| C4K68\_12090Tola\_1753 | 19.3 | 12.3 | 11.8 | 12.4 | 14.6 | 21.5 | 22.7 | 21 | 33.9 | 35.2 | 25.1 | 18.9 | 11 |  | 11.6 |
| Tola\_1753 | 12.1 | 17.1 | 15.4 | 13.5 | 11.6 | 11.6 | 9.8 | 10.3 | 9.4 | 8.5 | 10.3 | 10.7 | 50.4 | 11.6 |  |

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