**Figure S1.** LC-MS/MS coverage of the EAR28894 protein. Identified peptides mapped across the entirety of the sequence, with the exception of an N-terminal segment consisting of the first 13-27 amino acid residues. Residues highlighted in green represent chemical modifications (e.g., deamidation, oxidation).
Figure S2. Ratio of semi-tryptic to tryptic peptides across all proteins in LC-MS/MS data. EAR28894 deviates from all other proteins in this regard, and therefore is likely to be most heavily processed by non-trypsin proteases.
Figure S3. Pellicle biofilms formed by the WT vs Δslr4 mutant strain. Liquid cultures were grown for 8 hours (shaking) in liquid Difco marine broth media, followed by 72 hours of static (non-shaking) incubation.
Figure S4. TEM micrograph of a *P. tunicata* cell revealing the presence of a putative outermost capsular layer. Scale bar = 500 nm.
Figure S5. TEM micrographs of 48-hr pellicle biofilms (WT versus Δslr4 mutant strain). Top left – dense cell clusters in the WT strain interconnected by biofilm matrix material. Bottom left – a WT cell within a biofilm with extracellular biofilm matrix including fibrous structures coated by S-layer material. Top right – image taken from a Δslr4 biofilm showing a sparser cell distribution that is not connected by S-layer associated matrix material. Bottom right – two examples from the image above of deformed cell shapes. Scale bars are 2 microns, with the exception of the bottom left image where it is 100 nm.
Figure S6. Hypothetical model of Slr4 S-layer lattice assembly (right) compared to rsaA S-layer assembly (left). A smaller (truncated) S-layer subunit relative to rsaA could alter inter-subunit interactions to result in a four-fold symmetric pattern (right) instead of a six-fold hexagonal symmetry (left). The model is consistent with the top-down view of a square lattice as observed in Figs. 3 and 4, as well as the side-view showing V-shaped cups as shown in Fig. 4.
Table S1. Top proteins identified by LC-MS/MS.

| Accession     | -10lgP | Cov. (%) | #Peptides | #Unique | PTM | Avg. Mass (kDa) | Description                                                |
|---------------|--------|----------|-----------|---------|-----|-----------------|------------------------------------------------------------|
| EAR28894.1    | 468.71 | 93       | 111       | 110     | Y   | 59235           | hypothetical protein PTD2_07619                            |
| EAR29208.1    | 170.89 | 20       | 12        | 12      | Y   | 68830           | hypothetical protein PTD2_09189                            |
| EAR29563.1    | 133.95 | 8        | 3         | 3       | Y   | 60109           | flagellin                                                  |
|               |        |          |           |         |     |                 | TonB-dependent outer membrane receptor                     |
| EAR27917.1    | 104.01 | 5        | 4         | 4       | N   | 103265          | 30S ribosomal subunit protein S1                           |
| EAR28105.1    | 75.5   | 6        | 3         | 3       | N   | 61087           | 139223                                                     |
| EAR30644.1    | 56.2   | 0        | 2         | 2       | Y   | 8               | fibronectin type III domain protein                        |
| EAR27255.1    | 53.86  | 2        | 2         | 1       | N   | 67577           | putative lipoprotein                                        |
| EAR28194.1    | 50.54  | 3        | 2         | 2       | N   | 82187           | TonB-dependent receptor                                    |
|               |        |          |           |         |     |                 | sensor histidine kinase/response regulator                  |
| EAR26551.1    | 48.39  | 3        | 2         | 2       | Y   | 105969          |                                                            |
| EAR27983.1    | 44.82  | 3        | 1         | 1       | N   | 63152           | putative orphan protein                                    |
Table S2. Supporting peptides for EAR28894 identification by LC-MS/MS.

| Peptide | -10gP | Mass | L | ppm | m/z | z | RT | F | Scan | #Spec | Start | End | PTM |
|---------|-------|------|---|-----|-----|---|----|---|------|-------|-------|-----|-----|
| RYVMVEGVTAWQSLAAAGIK.D | 119.18 | 2031.025 | 19 | -7.4 | 1016.512 | 2 | 20.76 | 2 | 3156 | 9 | 491 | 509 |
| K.DGLEYTQATALEK | 117.52 | 2605.4 | 25 | -8.2 | 1303.697 | 2 | 22.33 | 2 | 3432 | 45 | 140 | 164 |
| KPIVAVGIGGKY.K | 111.23 | 2047.02 | 19 | -5.9 | 683.3431 | 3 | 20.02 | 2 | 3018 | 5 | 491 | 509 |
| RY.M(+15.99)VEGVI | 107.36 | 2418.162 | 23 | -4.7 | 1210.083 | 2 | 24.78 | 2 | 3863 | 13 | 517 | 539 |
| TAWQSLAAAGIK.D | 104.91 | 1469.722 | 14 | -6.2 | 735.8639 | 2 | 11.4 | 2 | 1422 | 4 | 477 | 490 |
| R.DMG( +15.99)GVT | 100.89 | 1535.765 | 14 | -4.9 | 768.8862 | 2 | 20.01 | 2 | 3017 | 4 | 413 | 426 |
| GSGDDAVATAFDGSG.R | 97.12 | 2403.069 | 27 | -7.9 | 802.0238 | 3 | 14.93 | 2 | 2057 | 9 | 86 | 112 |
| R.DM(+15.99)GLVDT | 92.65 | 2434.157 | 23 | -6.5 | 1218.078 | 2 | 20.99 | 2 | 3197 | 6 | 517 | 539 |
| DALGKEGGYDSSTTG.FK.V | 91.34 | 1631.852 | 15 | -6.5 | 816.9279 | 2 | 19.91 | 2 | Ta | 3 | 298 | 312 |
| R.QOFAN(+98)NLTA | 90.54 | 2191.189 | 21 | -7.8 | 1096.593 | 2 | 19.65 | 2 | 2941 | 18 | 540 | 560 |
| DVLAV.K.H | 88.79 | 1551.76 | 14 | -6.3 | 776.8825 | 2 | 18.42 | 2 | 2717 | 3 | 413 | 426 |
| K.TSVTATTAVLQQT | 88.03 | 1728.019 | 17 | -8.1 | 865.0095 | 2 | 19.96 | 2 | 3005 | 2 | 148 | 164 |
| AVK.H | 88 | 2032.009 | 19 | -3.6 | 1017.008 | 2 | 21.43 | 2 | 3275 | 3 | 491 | 509 |
| R.YVMVEGVTAWQ(+98)SLAAAGIK.D | 87.8 | 2131.083 | 22 | -5.6 | 1066.543 | 2 | 22.34 | 2 | 3433 | 4 | 352 | 373 |
| K.LSSAELMAANV.LAGGDDTV.K | 87.28 | 2606.384 | 25 | 10.3 | 652.61 | 4 | 22.38 | 2 | 3441 | 12 | 140 | 164 |
| D.GLEYTQATALEK | 84.32 | 1630.868 | 15 | -3.8 | 816.4381 | 2 | 20.18 | 2 | 3053 | 4 | 298 | 312 |
| K.SVTATTAVLQQT | 83.11 | 1861.016 | 19 | -7.8 | 931.5978 | 2 | 18.03 | 2 | 2646 | 3 | 28 | 46 |
| ALGKEGGYDSSTTG.FK.V | 83.06 | 1174.627 | 11 | -5.3 | 588.3175 | 2 | 23.81 | 2 | 3689 | 7 | 517 | 527 |
| K.AEDLTIQFSGAK.L | 82.59 | 1413.678 | 13 | -4.9 | 707.8427 | 2 | 19.86 | 2 | 2984 | 31 | 165 | 177 |
| K.LAAFYGVSLTK.A | 82.53 | 1239.686 | 12 | -6.6 | 620.8463 | 2 | 19.91 | 2 | 2993 | 2 | 74 | 85 |
| K.LSSAELM(+15.99)AANVAVL | 81.88 | 2147.078 | 22 | 0.2 | 1074.546 | 2 | 19.61 | 2 | 2933 | 4 | 352 | 373 |
| VK.S | 81.48 | 2768.474 | 27 | -5.8 | 693.1218 | 4 | 16.99 | 2 | 2429 | 5 | 113 | 139 |
| R.LTTVHHTAANAC(+57.02)LATLVPKLS | 81.46 | 1631.852 | 15 | -6.5 | 816.9279 | 2 | 20.55 | 2 | 3119 | 2 | 298 | 312 |
| T.TAAK.D | 455 | 296 | 352 | 140 | 413 | 2717 | 426 | 296 |
| R.QL(+98)QANNTLTA | 80.18 | 1901.996 | 17 | -4.4 | 952.001 | 2 | 19.83 | 2 | 2976 | 4 | 296 | 312 |
| DVLAV.K.H | 79.7 | 2322.321 | 22 | 3.3 | 775.1131 | 3 | 23.76 | 2 | 3680 | 3 | 455 | 476 |
| K.VLAEVNTVPKSK | 79.29 | 2323.294 | 22 | -4.5 | 775.4351 | 3 | 24.08 | 2 | 3738 | 7 | 455 | 476 |
| T.TAAK.D | 77.2 | 1254.718 | 12 | -3.7 | 419.2451 | 3 | 16.45 | 2 | 2334 | 13 | 540 | 551 |
| R.VN(+98)GSGVFGT | 76.07 | 2154.001 | 24 | -5.8 | 719.0035 | 3 | 17.55 | 2 | 2553 | 4 | 51 | 74 |
| AADATNSAANCA.L | 75.15 | 1867.961 | 18 | -2.8 | 934.9854 | 2 | 20.84 | 2 | 3171 | 2 | 492 | 509 |
| Y.MVEGVTAWQSLAAGIK.D | 74.87 | 1470.706 | 14 | -5.4 | 736.3564 | 2 | 11.78 | 2 | 1499 | 3 | 477 | 490 |
| R.JHGLTFK.T | 74.7 | 3059.577 | 31 | -8.3 | 1020.858 | 3 | 25.6 | 2 | 4007 | 17 | 183 | 213 |
| H.LAAADSGSGDVA | 74.54 | 1779.802 | 20 | -7.6 | 890.9016 | 2 | 14.95 | 2 | 2061 | 2 | 93 | 112 |
| TEADGSGR.E | 73.72 | 1757.971 | 18 | -6.2 | 879.9873 | 2 | 15.75 | 2 | 2208 | 4 | 122 | 139 |
| A.ANACLAVKPKVL | 71.1 | 814.470 | 7 | 0.2 | 408.2424 | 2 | 14.68 | 2 | 2009 | 5 | 569 | 575 |
| Length | Fraction | Oxidation | Deamidation | Carbamidomethylation |
|--------|----------|-----------|-------------|----------------------|
| I. HLGTFK.T | 31.06 | 701.386 | 6 | -3.1 | 351.6992 | 2 | 14.66 | 2 | 2005 | 2 | 570 | 575 |
| R. GNGILELSNIFLDS TGLAATTIVSVM(+15.9) 9 | 30.98 | 3632.788 | 36 | -7.2 | 1211.928 | 3 | 27.78 | 2 | 4376 | 1 | 228 | 263 | 1 |
| 9 V NSFGTNTS GTK.F K. AEDTLTFTQ(+.98) F SGK. L | 30.85 | 1414.662 | 13 | 9.7 | 708.345 | 2 | 25.55 | 2 | 3999 | 3 | 165 | 177 | 2 |
| T. TPAN(+.98) DFVR. G | 29.92 | 919.4399 | 8 | -1.2 | 460.7267 | 2 | 13.37 | 2 | 1778 | 1 | 220 | 227 | 2 |
| V. ALEVVTNVPK. D GSGDVATFADGS G.R.E H. TNAGTQGTDITVR. Y | 28.96 | 1295.574 | 14 | -11.1 | 648.7872 | 2 | 13.77 | 2 | 1845 | 1 | 99 | 112 |
| | 28.71 | 1042.53 | 10 | -4.7 | 522.2696 | 2 | 12.13 | 2 | 1564 | 1 | 113 | 122 |
| R. ELTTVHTTA. N R. DM(+15.99) GLVT D A L G G E G Y D S | 27.7 | 1711.798 | 16 | -8.4 | 856.8988 | 2 | 21.26 | 2 | 3246 | 1 | 517 | 532 | 1 |
| K. I T A E G Q D R H I L G T F K. T R. Y M(+15.99) V E G V H T A W Q. S A. T V K P V L S T T A A K. D | 27.54 | 1684.89 | 15 | -4.1 | 422.2269 | 4 | 15.54 | 2 | 2167 | 1 | 561 | 575 |
| R. DM(+15.99) GLVT D A L G G E G Y D S K. I T A E G Q D R H I L G T F K. T R. Y M(+15.99) V E G V H T A W Q. S A. T V K P V L S T T A A K. D | 26.23 | 1241.723 | 12 | -9.6 | 608.3632 | 2 | 14.35 | 2 | 1949 | 1 | 128 | 139 |
| K. N P T S A N V L P A N T T Y. V T. N P M I S V Q D F T V K. V | 26.21 | 1560.778 | 15 | -11.4 | 781.3875 | 2 | 17.88 | 2 | 2619 | 1 | 316 | 330 |
| K. T S V T A T T A V L Q Q T A I G T A K A H A K K. A D G T A A H A V A A D . G | 26.18 | 1214.723 | 12 | -7.5 | 689.8502 | 2 | 20.05 | 2 | 3026 | 1 | 415 | 426 |
| D. I L D I T D S Q R. F K. I T N (+.98) A G T Q (+.98) T D I T V R. Y K. G T N P M(+15.99) J S V Q(+.98) D F T V K. V | 25.55 | 1172.64 | 10 | -7 | 587.3232 | 2 | 17.34 | 2 | 2502 | 2 | 204 | 213 |
| D. I L D I T D S Q R. F K. I T N (+.98) A G T Q (+.98) T D I T V R. Y K. G T N P M(+15.99) J S V Q(+.98) D F T V K. V | 25.35 | 1471.69 | 14 | -0.1 | 736.8524 | 2 | 12.34 | 2 | 1601 | 1 | 477 | 490 | 2 |
| V. F V Y G G A K. I R. E L T V H T T A A N (+.98) A C (+57.02) L | 24.66 | 740.3857 | 7 | -5.8 | 371.198 | 2 | 15.66 | 2 | 2190 | 1 | 554 | 560 |

* L = Length; F = Fraction; 1 = Oxidation (M); 2 = Deamidation (NQ); 3 = Carbamidomethylation
| Protein sequence (accession #) | Species | Marine Host-associated | Fresh water | Habitat description | Reference/Source |
|--------------------------------|---------|------------------------|-------------|--------------------|------------------|
| WP_055732151.1 | Agarivorans gilvus | Yes | Yes | Seaweed | https://www.ncbi.nlm.nih.gov/pubmed/20369530 |
| WP_026972292.1 | Agarivorans marinus | Yes | | Seawater | https://www.dsmz.de/catalogues/details/culture/DSM-23064.html |
| WP_026957844.1 | Agarivorans taiwanensis | Yes | | Seawater | https://www.ncbi.nlm.nih.gov/pubmed/19567569 |
| WP_091340846.1 | Alkalimonas amylolytica | No | Yes | Lake Chahannor in China | https://www.ncbi.nlm.nih.gov/pubmed/14986177 |
| WP_124748942.1 | Alteromonas facilis | Yes | Yes | Isolated from a sea cucumber culture pond in China Isolated from the alkaline, low-saline ikaita columns in the Ilka Fjord, SW Greenland | https://www.ncbi.nlm.nih.gov/pubmed/30526643 |
| WP_046556214.1 | Arsukibacterium ikkense | Yes | | Seawater samples from the Chukchi Sea in the Arctic Ocean | https://www.ncbi.nlm.nih.gov/pubmed/16790334 |
| WP_085282491.1 | Colwellia chukchiensis | Yes | | Isolated from seawater samples from the mussel Mytilus edulis from the South Sea in Korea | https://www.ncbi.nlm.nih.gov/pubmed/20495042 |
| WP_085298075.1 | Colwellia mytili | Yes | Yes | Seawater Marine sediment, Pacific Ocean: the Tonga Trench marine sediment metagenome; deep-sea hydrothermal vent sediments from dive 4571_4 depth 0-3 cm | https://www.ncbi.nlm.nih.gov/pubmed/27902189 |
| WP_118961217.1 | Colwellia sp. RSH04 | Yes | | Seawater | https://www.ebi.ac.uk/biosamples/samples/SAMN09916314 |
| WP_057830656.1 | Colwellia sp. TT2012 | Yes | | | https://www.ncbi.nlm.nih.gov/pubmed/20495042 |
| RL6B9876.1 | Deltaproteobacteria bacterium | Yes | | | https://www.ncbi.nlm.nih.gov/protein/RLB69876.1 |
| PCH94196.1,PCI39289.1 | Gammaproteobacteria bacterium | Yes | | Mediterranean seawater-France isolated from sea-ice cores collected from coastal areas of eastern Antarctica | https://www.uniprot.org/taxonomy/1805126 |
| WP_040521162.1, WP_070111740.1 | Glaciecola punicea | Yes | | | https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.022970-0 |
| GAB56998.1 | Glaciecola punicea ACAM 611 | Yes | | Antarctic sea ice | https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.000619 |
| PTB83247.1,PTB83248.1,PTB83653 | Idiomarina aestuarii | Yes | | Isolation of the type strain from shallow coastal seawater. | https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.002970-0 |
| 0.1,PTB835125.1,PTB835126.1,UR04164 | Idiomarina aquatica | Yes | | Isolated from salterns isolated from the reef-building coral Isopora palifera | https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.035592-0 |
| 1646.1,UR04164 | Idiomarina aquimaris | Yes | Yes | | https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.000619 |
| 7.1,UR041648.1 | Idiomarina atlantica | Yes | | Isolated from the deep sea sediment of the North Atlantic Ocean | https://link.springer.com/article/10.1007/s210482014-0337-7 |
| GenBank Accession | Species | Isolated From | Description |
|-------------------|---------|---------------|-------------|
| RUO53158.1, WP126763865.1, WP126763868.1, WP126763869.1, WP13056263.1, WP126771356.1, WP126771358.1, WP126771362.1 | Idiomarina halophila | Yes | isolated from the saltpond located in Gomso, Republic of Korea |
| RUO62606.1, WP126753970.1 | Idiomarina homiensis | Yes | isolated from seashore sand in Korea |
| WP_026861598.1, WP_126775152.1 | Idiomarina sediminum | Yes | isolated from a sea salt evaporation pond on the Island of Sal in the Cape Verde Archipelago |
| MBG23301.1 | Idiomarina insulisalsae | Yes | isolated from a sea salt evaporation pond on the Island of Sal in the Cape Verde Archipelago |
| WP_088331539.1 | Idiomarina sp. | Yes | marine metagenome from a Mediterranean Sea water sample |
| WP_031571199.1 | Parachaeoarchaeum t covidensis | No | Yes | USA: Spring Lake; San Marcos; Texas marine metagenome from a South Atlantic Ocean water sample |
| MBU77382.1 | Pseudoalteromonas nudaicae | Yes | marine metagenome from a South Atlantic Ocean water sample |
| WP_077560941.1, WP_010361438.1 | Pseudoalteromonas norvegica | Yes | Sea of Japan, Pacific Ocean marine metagenome from a South Atlantic Ocean water sample |
| WP_091983032.1 | Pseudoalteromonas denitrificans | Yes | Marine |
| SFC52264.1 | Pseudoalteromonas denitrificans DSM 6059 | Yes | Marine |
| KID36130.1 | Pseudoalteromonas ealykovi | Yes | Marine |
| CCQ10312.1, WP_010368594.1, WP_045962959.1, WP_045987911.1, WP_088531627.1, WP_117332793.1 | Pseudoalteromonas piscicida | Yes | Marine |
| WP_119852593.1 | Pseudoalteromonas profundis | Yes | Marine |
| WP_022946007.1 | Pseudoalteromonas ruthenica | Yes | Marine |
| WP_130050461.1 | Pseudoalteromonas shioyasakiensis | Yes | Marine |
| MAD02172.1 | Pseudoalteromonas sp. | Yes | Marine |
| MBD58444.1 | Pseudoalteromonas sp. | Yes | Marine |
| Accession | Taxonomy | Isolated From | Source | Reference |
|-----------|----------|---------------|--------|-----------|
| WP_042150686.1 | *Pseudoalteromona* sp. '520P1 No. 412' | Yes | Marine | https://mra.asm.org/content/2/6/e01346-14 |
| WP_042150686.1 | *Pseudoalteromona* sp. '520P1 No. 423' | Yes | Marine | Isolated from gut of comb jelly | https://www.uniprot.org/proteomes/UP000941313 |
| WP_0690000079.1 | *Pseudoalteromona* sp. BMB | Yes | Octocoral | https://onlinelibrary.wiley.com/doi/full/10.1002/jobm.201800087 |
| WP_130151616.1 | *Pseudoalteromona* sp. CO133X | Yes | Octocoral | Isolated from Northern Yellow Sea | https://www.ncbi.nlm.nih.gov/nuccore/NZ_NHNM00000000.1 |
| WP_130151616.1 | *Pseudoalteromona* sp. CO302Y | Yes | Host-associated (Muricea sp.), Panama: Coiba National Park | https://www.ncbi.nlm.nih.gov/nuccore/NZ_RCSQ00000000.1 |
| WP_099029058.1 | *Pseudoalteromona* sp. GCY | Yes | Isolated from surface of crustose coralline alga | https://www.ncbi.nlm.nih.gov/nuccore/NZ_RJHW00000000.1 |
| WP_125251093.1 | *Pseudoalteromona* sp. CO342X | Yes | Isolated from surface saline water | Isolation source (ocean), host - Hymeniacidon perleve (marine sponge) | https://www.ncbi.nlm.nih.gov/nuccore/NZ_AKXJ00000000.1 |
| WP_086997608.1 | *Pseudoalteromona* sp. JB197 | No | Isolated from cheese rind | https://www.ncbi.nlm.nih.gov/nuccore/NZ_NRGZ00000000.1 |
| WP_119861005.1 | *Pseudoalteromona* sp. MSK9-3 | Yes | Isolated from surface saline water | https://www.ncbi.nlm.nih.gov/nuccore/NZ_MJET00000000.1 |
| WP_017217139.1 | *Pseudoalteromona* sp. NJ631 | Yes | Isolated from host: Neogoniolithon solubile, USA: reef near Looe Key | https://www.ncbi.nlm.nih.gov/nuccore/NZ_BCSQ00000000.1 |
| WP_128731076.1 | *Pseudoalteromona* sp. J010 | Yes | Seawater (Indian Ocean) | https://www.ncbi.nlm.nih.gov/nuccore/NZ_SAMD00039894 |
| WP_053910113.1 | *Pseudoalteromona* sp. SW0106-04 | Yes | Marine | Isolated from gut of comb jelly | https://www.uniprot.org/proteomes/UP000941313 |
| Genbank Accession | Species | Isolated From | Original Isolation | Source Link |
|-------------------|---------|---------------|--------------------|-------------|
| WP_105171055.1    | Pseudoalteromonas sp. T1lg24 | Isolated from sea water (sediment depth) | Yes | [https://www.ncbi.nlm.nih.gov/nuccore/NZ_PQCB00000000.1](https://www.ncbi.nlm.nih.gov/nuccore/NZ_PQCB00000000.1) |
| WP_024611180.1    | Pseudoalteromonas sp. TB64 | Isolated from a sponge, Antarctica | Yes | [https://www.ncbi.nlm.nih.gov/nuccore/NZ_AUTQ00000000.1](https://www.ncbi.nlm.nih.gov/nuccore/NZ_AUTQ00000000.1) |
| WP_010561444.1,  | Pseudoalteromonas sp. spongiae | Isolated from the surface of the sponge Mycale adhaerens in Hong Kong waters | No, Yes | [https://www.ncbi.nlm.nih.gov/pubmed/16014487](https://www.ncbi.nlm.nih.gov/pubmed/16014487) |
| WP_009838156.1,  | Pseudoalteromonas sp. tunicata | Marine organism originally isolated from tunicates | Yes | [https://www.ncbi.nlm.nih.gov/pubmed/9828422](https://www.ncbi.nlm.nih.gov/pubmed/9828422) |
| WP_009838156.1,  | Pseudoalteromonas sp. ulvae | Isolated from surface of marine alga | Yes | [https://www.ncbi.nlm.nih.gov/pubmed/11491351](https://www.ncbi.nlm.nih.gov/pubmed/11491351) |
| WP_134053939.1    | Rheinheimera aquimartis | Isolated from seawater of the East Sea in Korea | Yes | [https://www.ncbi.nlm.nih.gov/pubmed/17625162](https://www.ncbi.nlm.nih.gov/pubmed/17625162) |
| WP_019674711.1    | Rheinheimera perlucida | Isolated from surface water from Baltic Sea | Yes | [https://www.ncbi.nlm.nih.gov/pubmed/16957117](https://www.ncbi.nlm.nih.gov/pubmed/16957117) |
| WP_132584183.1    | Rheinheimera sp. D18 | Isolated from yellow sea (China) | Yes | [https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP037745.1](https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP037745.1) |
| WP_127697655.1    | Rheinheimera sp. KYP3 | Isolated from freshwater stream | No, Yes | [http://link-springer-com-443.webvpn.jxust.edu.cn/article/10.1007%2Fs00203-019-01657-5](http://link-springer-com-443.webvpn.jxust.edu.cn/article/10.1007%2Fs00203-019-01657-5) |
| WP_068063761.1    | Rheinheimera sp. SA_1 | Isolated from "iron backwash sludge of a waterworks in Germany" | No, Yes | [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4991719/](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4991719/) |
| Accession   | Name                        | Isolated from                  | Source Information                                                                 |
|------------|-----------------------------|--------------------------------|------------------------------------------------------------------------------------|
| PHS21107.1 | Robiginitomaculum sp.       | from Antarctic seawater        | http://www.bacterio.net/robiginitomaculum.html                                      |
| WP_10933887.1 | Salinimonas sp. HMF8227   | Isolated from saltern in South Korea | https://www.ncbi.nlm.nih.gov/nuccore/NZ_CP029347.1                              |
| WP_11997519.1 | Shewanella algidipiscicola | isolated from marine fish of the Danish Baltic Sea | https://www.ncbi.nlm.nih.gov/pubmed/17267977                                      |
| WP_05974416.1 | Shewanella frigidermarina | Isolated from Antarctic coastal areas | https://www.ncbi.nlm.nih.gov/pubmed/11837303                                      |
| WP_10894712.1 | Shewanella halifaxensis   | Isolated from marine sediment  | https://www.ncbi.nlm.nih.gov/pubmed/16403888                                       |
| WP_11540597.1 | Shewanella putrefaciens   | Associated with spoiled fish   | https://www.ncbi.nlm.nih.gov/pubmed/2641275                                       |
| WP_01214335.1 | Shewanella sediminis       | Isolated from marine sediment  | https://www.ncbi.nlm.nih.gov/pubmed/1604474                                        |
| WP_07641100.1 | Shewanella sp. UCD-KL12   | Isolated from seagrass         | https://www.ncbi.nlm.nih.gov/pubmed/28360178                                       |
| WP_02877174.1 | Shewanella waksmanii      | Marine strain isolated from sipuncula | https://www.ncbi.nlm.nih.gov/pubmed/13130035                                      |
| WP_04483070.1 | Thalassomonas actiniarum  | Isolated from marine animals   | https://www.ncbi.nlm.nih.gov/pubmed/19325582                                       |
| WP_04483657.1 | Thalassomonas viridans     | isolated from oysters off the Mediterranean coast | https://www.ncbi.nlm.nih.gov/pubmed/11491324                                      |
| WP_11599956.1, WP_11600723.1, WP_116007235.1, WP_116014431.1 | Thalassotalea euphylliae | Isolated from coral             | https://www.ncbi.nlm.nih.gov/pubmed/27582443                                      |
| WP_074500610.1 | Thalassotalea sp. PP2-459 | Isolated from clam larvae in shellfish hatchery in Spain | https://www.ncbi.nlm.nih.gov/pubmed/23743010                                      |
| Accession   | Species             | Is Pathogen | Isolated From | Source Link |
|-------------|---------------------|-------------|---------------|-------------|
| WP_074191941.1 | *Vibrio antiquarius* | Yes          | Isolated from deep sea hydrothermal vent | https://www.pnas.org/content/112/21/E281 |
| WP_104968991.1 | *Vibrio diabolicus* | Yes Yes      | Isolated from a deep-sea hydrothermal vent annelid worm | https://www.ncbi.nlm.nih.gov/pubmed/9336897 |
| WP_005434363.1 | *Vibrio harveyi* | Yes Yes      | Pathogen of marine vertebrates and invertebrates | https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1472-765X.2006.01989.x |
| WP_038864853.1, WP_045422135.1 | *Vibrio jasicida* | Yes Yes      | Isolated from marine vertebrates and invertebrates | https://www.ncbi.nlm.nih.gov/pubmed/21984666 |
| WP_088881413.1 | *Vibrio rotiferianus* | Yes Yes      | Marine pathogen | https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3133291/ |
| WP_095760150.1 | *Vibrio sp. V1B* | Yes Yes      | Isolated from gut of saltwater clam | https://www.ncbi.nlm.nih.gov/pubmed/29051252 |
Table S4. Oligos used for plasmid construction

| Oligo ID | Sequence                                                                 |
|----------|---------------------------------------------------------------------------|
| JC467    | TCAGTTTCATGAAAACCTGGGAGAATGCTGAGTAAGCAACCGCTAAGTTTAAGTGT                  |
| JC468    | ACTACTAAAACCTTAGCGGTTGTCTTTACTGCAATTCATTCTCCAAGTTCATGAAACTGA            |
| JC470    | CGGCAAGGCTTAAACTTTGCTGCGTT                                               |
| JC491    | GCAGGATCCAAACAAATTATTTTAGGTTGTTAATTAG                                    |
| JC492    | GCAGGAAGCCTTGCGGATTAATGGACACAGTGTCAAC                                   |
Supplementary Text

>Protein PTD2_07619 (EAR28894)
MEIMFKTLALLAİTÇVSVAAANAVKTSVTATAVLQQTAIGTAKAHAKGTLGASGVFGTAADATNSANCKALAA FYGVSLTKAĐCTAAHAVAADGSGDVTADFSGRELÀTTVHANACLYKPVLSSTTAADKGLEYQTATALEIKPV VIVAGIGGYKAEDLTIFQFSAGKDLTTKTTAPSTVIVAAGAAGAVFĐIDLĐTĐQRIFTKVATTTPANDFVRGNI LELESNIFLDSTGLAATSVMSAVKSFVATĐSATIVSLLPQYTTEVTTLSDFIVĐVDKQRQFANNLTADV LAVKHTKNSANVLPANTTVVTVGDŚWVAPSVTDNKDGKLSSAELMAANVLAGGĐTVSKLALNATNTLETVNIVGALĐANTÍTFTVFYGĐSGKGTNPMSIȘQDVČTVKDĐMSKSVKAVNSŁAKTAAGTWKLNŚVČVY VYVPFGPÁTQFJLRHólnAGTQGDĐTIVYMVEVHAGWQALSAAIKĐAPKGVĐMLGLVTDĐLGEĐGĐTSTTTFK VÀLEVDÖNSKDVFGVYAGKITAEGQDRHĠTFFKTNNV

> PTD2_07619 (EAR28894) deletion/replacement
MHQ

> PTD2_07619 (EAR28894) gene
ATGGAAATTATGTTGCTCAAGAAGACCTCAGTACAGCAATCTAGTCTTGTTTCTCTGTTAGCAGCTAAATGCATCTGCT TTTTATCTGGTTTCTATTAACTAAGCTGATGCTACAGTCTGCTACCTGTCGGTACAGCTGCTACGCTAATCTTGGTGC TAAÅCCAGTCTCCTACCTACAGTGCTAAGATGCTTTCTTGTGTTAGCTAAGCTAAGCTGCTTAATACTAAAAATGCTGCT TTTTATCTCATCGTTCTAGTTTAAGATGCTTTCTTGTGTTAGCTAAGCTAAGCTGCTTAATACTAAAAATGCTGCT TTTTATCTCATCGTTCTAGTTTAAGATGCTTTCTTGTGTTAGCTAAGCTAAGCTGCTTAATACTAAAAATGCTGCT

> PTD2_07619 (EAR28894) deletion/replacement
ATGCT

ATGCTGAGTAA