**Supplementary Table 1. Strains used in this study**

|  |  |  |
| --- | --- | --- |
| Strain |  | Reference/source |
| *Escherichia coli* strains | | |
| DH5α | F−,ø80d*lacZ*ΔM15,Δ(*lacZYA-argF*) U169 *deoR*, *recA*1, *endA*1, *hsdR*17 (rk-,mk+), *phoA*, *supE*44, ʎ-, *thi*-1, *gyrA*96, *relA*1 - | (Hanahan 1983) |
| DH5α λpir | *sup* *E44*, Δ*lacU169* (Φ*lacZ*ΔM15), *recA1*, *endA1*, *hsdR17*, *thi-1*, *gyrA96*, *relA1*, λ*pir* | (Platt et al 2000) |
| SM10 λpir | *thi thr leu tonA lacY supE recA*::RP4-2 Tc::Mu Km ʎ*pir* | (R et al 1983) |
| *Vibrio cholerae* strains | | |
| co969 | new El Tor clinical strain isolated in India (after the re-establishment of O1 dominance after O139; SmR), HapR- | MKW collection |
| co907 | clinical isolate, HapR-, SmR | MKW collection |
| co943 | clinical isolate, HapR-, SmR | MKW collection |
| o139 | clinical isolate, HapR-. SmR | MKW collection |
| vc151 | clinical isolate, HapR-, SmR | MKW collection |
| vc208 | clinical isolate, HapR-, SmR | MKW collection |
| A1552 | O1 El Tor, Inaba, RifR | (Yildiz and Schoolnik 1998) |
| A1552\* | O1 El Tor, Inaba, RifR SmR | This work |
| C6706 | O1 El Tor strain isolate from Peru; SmR | (Thelin and Taylor 1996) |
| N16961 | O1 El Tor strain isolate from Bangladesh; SmR | (Heidelberg et al 2000) |
| co969 Δ*lacZ* | co969, Δ*lacZ* clean deletion mutant; SmR | This work |
| co969:*hapRc* | co969 with its native *hapR* exchanged for *hapR* of C6706 (*hapR*c); SmR | This work |
| co969:*hapRc* Δ*bipA* | co969 with its native *hapR* exchanged for *hapR* of C6706 (*hapR*c), Δ*bipA* clean deletion mutant; SmR | This work |
| co969 Δ*bipA* | co969, Δ*bipA* clean deletion mutant; SmR | This work |
| co969 Δ*vpsT* | co969, Δ*vpsT* clean deletion mutant; SmR | This work |
| co969 Δ*vpsT* Δ*bipA* | co969, Δ*vpsT* andΔ*bipA* clean deletion mutants; SmR | This work |
| co969 Δ*vpsR* | co969, Δ*vpsR* clean deletion mutant; SmR | This work |
| co969 Δ*vpsR* Δ*bipA* | co969, Δ*vpsR* andΔ*bipA* clean deletion mutants; SmR | This work |
| co969 Δ*vqmA* | co969, Δ*vqmA* clean deletion mutant; SmR | This work |
| co969 Δ*vqmA* Δ*bipA* | co969, Δ*vqmA* andΔ*bipA* clean deletion mutants; SmR | This work |
| co969:*bipA*-flag | co969 with its native *bipA* exchanged for *bipA-*flag; SmR | This work |
| C6706 Δ*hapR* | C6706, Δ*hapR* clean deletion mutant; SmR | This work |
| A1552 Δ*hapR* | A1552, Δ*hapR* clean deletion mutant; SmR | This work |
| co969 Δ*lonA* | co969, Δ*lonA* clean deletion mutant; SmR | This work |

**Supplementary Table 2. Plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| Plasmid | Relevant properties | Reference/source |
| pSC189 | Himar 1 suicide transposon vector; ApR, KmR | (Chiang and Rubin 2002) |
| pCB192N | β-galactosidase promoter-probe vector; ApR | (Kimsey and Waldor 2009) |
| pCB192N-P*vpsR* | pCB192N carrying the P*vpsR* promoter; ApR | This work |
| pCB192N-P*vpsT* | pCB192N carrying the P*vpsT* promoter; ApR | This work |
| pCB192N-P*vpsL* | pCB192N carrying the P*vpsL* promoter; ApR | This work |
| pCB192N-P*vpsU* | pCB192N carrying the P*vpsU* promoter; ApR | This work |
| pCB192N-P*bap1* | pCB192N carrying the P*bap1* promoter; ApR | This work |
| pCB192N-P*gyrA* | pCB192N carrying the P*gyrA* promoter; ApR | This work |
| pCB192N-P*hfq* | pCB192N carrying the P*hfq* promoter; ApR | This work |
| pCB192N-P*mrcA* | pCB192N carrying the P*mrcA* promoter; ApR | This work |
| pCVD442 | oriR6K, *sacB* containing suicide vector for double homologous recombination; ApR | (Donnenberg and Kaper 1991) |
| pCVD442 Δ*lacZ* | pCVD442-based suicide plasmid for clean deletion of *lacZ*; ApR | This work |
| pCVD442 Δ*bipA* | pCVD442-based suicide plasmid for clean deletion of *bipA*; ApR | This work |
| pCVD442-Δ*vpsR* | pCVD442-based suicide plasmid for clean deletion of *vpsR*; ApR | This work |
| pCVD442-Δ*vpsT* | pCVD442-based suicide plasmid for clean deletion of *vpsT*; ApR | This work |
| pCVD442-Δ*hapR* | pCVD442-based suicide plasmid for clean deletion of *hapR*; ApR | This work |
| pCVD442-Δ*vqmA* | pCVD442-based suicide plasmid for clean deletion of *vqmA*; ApR | This work |
| pCVD442-*hapRc* | pCVD442-based suicide plasmid for chromosomal exchange of *hapR* to *hapR* from C6706 (*hapR*c); ApR | This work |
| pCVD442 Δ*lonA* | pCVD442-based suicide plasmid for clean deletion of *lonA;* ApR | This work |
| pHL100 | Replicating plasmid used for expressing genes under control of the IPTG-inducible P*lac* promoter; KmR | (Cava et al 2011) |
| pHL100-*bipA* | *bipA* from *V. cholerae* co969 inserted into BamHI-HindIII of pHL100; KmR | This work |
| pHL100-EC*bipA* | *bipA* from *E. coli* MG1655 K-12 inserted into EcoRI-HindIII of pHL100; KmR | This work |
| pHL100-PP*bipA* | *bipA* from *P. putida* KT2440 K-12 inserted into BamHI-HindIII of pHL100; KmR | This work |
| pHL100-*lonA* | *bipA* from *V. cholerae* co969 inserted into BamHI-HindIII of pHL100; KmR | This work |
| pET22b (+) | Expression vector with T7 promoter and terminator flanking MCS, *pelB* leader sequence for potential periplasmic localization and optional C-terminal His-tag sequence; AmpR | Novagen |
| pET22b-*bipA*-His | *bipA*-His inserted into NdeI-HindIII of pET22b(+) | This work |

**Supplementary Table 3. Primers used in this study**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| FCP | Primer | Sequence (5’ → 3’) | Restriction sites | Use |
| FCP  2523 | *vpsA* qPCR fw | GCAAGAAAACTACAACGCAGA |  | qRT-PCR *vpsA* |
| FCP  2524 | *vpsA* qPCR rev | CTCAAAGCCACCACCAAAAC |  |
| FCP  2521 | *vpsU* qPCR fw | TATCCAAGTGCGTAGTGCTG |  | qRT-PCR *vpsU* |
| FCP  2522 | *vpsU* qPCR rev | CACTTGCTGGACTGGGTTG |  |
| FCP  2525 | *vpsL* qPCR fw | CAATGTGTTGCAAGGAAGTATG |  | qRT-PCR *vpsL* |
| FCP  2526 | *vpsL* qPCR rev | CACTCGCTTCTCCATCTTGT |  |
| FCP  2527 | *rbmA* qPCR fw | GCGGAAGTGGATTGTGAGTT |  | qRT-PCR *rbmA* |
| FCP  2528 | *rbmA* qPCR rev | CGCTTTGGCTGGGAAGTAG |  |
| FCP  2529 | *rbmC* qPCR fw | GGGAAATCAACAACACAGCA |  | qRT-PCR *rbmC* |
| FCP  2530 | *rbmC* qPCR rev | AAATCCTGAACGAACCGAGA |  |
| FCP  2531 | *bap1* qPCR fw | TCTACCAGCGTTTTTCATCCT |  | qRT-PCR *bap1* |
| FCP  2532 | *bap1* qPCR rev | GTCAGCCACATCTTTCTCATC |  |
| FCP  2533 | *vpsR* qPCR fw | GCCTTGAAAGCGAGCTGT |  | qRT-PCR *vpsR* |
| FCP  2534 | *vpsR* qPCR rev | CGTCTCCACAGTCCCTTCTT |  |
| FCP  2535 | *vpsT* qPCR fw | CAGATTGTTGAAAGAGGCGTTAG |  | qRT-PCR *vpsT* |
| FCP  2536 | *vpsT* qPCR rev | TCGGTCAAAACATCATCAGAA |  |
| FCP  2539 | *gyrA* qPCR fw | GTTATCGTGGGTCGTGCTCT |  | qRT-PCR *gyrA* |
| FCP  2540 | *gyrA* qPCR rev | AGGCTTTGTTCCAGTCGTTG |  |
| FCP  2541 | *hfq* qPCR fw | TGCAAGGTCAGATCGAATCAT |  | qRT-PCR *hfq* |
| FCP  2542 | *hfq* qPCR rev | TGGTGGCTAACTGGACGAG |  |
| FCP  2519 | VC2744 qPCR fw | CGTAGGTGTTATCGGTGTTGG |  | qRT-PCR *bipA* |
| FCP  2520 | VC2744 qPCR rev | CGGTGGCTTGGTCAGTTT |  |
| FCP  2537 | *vqmA* qPCR fw | TAAGCAAGGTTTTCGGGATG |  | qRT-PCR *vqmA* |
| FCP  2564 | *vqmA* qPCR fw b | AACGGTGGAAGGGTATGAGG |  |
| FCP  799 | *lacZ P1* | CGGTCTAGAGCAAAGGCGTTATTG | XbaI | *lacZ* clean deletion mutant  (pCVD442 Δ*lacZ*) |
| FCP  800 | *lacZ P2* | TTTTTTGCGGCCGCTTTTTTCTCGGCAGGCAAGCGGCTATC |  |
| FCP  801 | *lacZ P3* | AAAAAAGCGGCCGCAAAAAACTGAATGTTGAGGTGATGCTGTG |  |
| FCP  802 | *lacZ P4* | CGGTCTAGAGAGCAAAAATTCAGGGTG | XbaI |
| FCP  803 | *lacZ check fw* | GGTGAGTGGTTCACAGAATCGGTG |  |
| FCP  804 | *lacZ check rev* | CTCAGTTGGCTCTTGCTTTGGCAAC |  |
| FCP  805 | *lacZ check-2 fw* | GCTGGATCGCACGTTGGCATG |  |
| FCP  806 | *lacZ check-2 rev* | CGGCTTGCGGTAGAGATACACATC |  |
| FCP  1533 | VC2744-P1 | GGCAGAGCTCTGGTGCTGGAATGTTTG | SacI | *bipA* clean deletion mutant  (pCVD442 Δ*bipA*) |
| FCP  1534 | VC2744-P2 | TTTTTTGCGGCCGCTTTTTTCGGGGTTTGCTTCACTTTTTCATTG |  |
| FCP  1535 | VC2744-P3 | AAAAAAGCGGCCGCAAAAAAATCACTGACTTTCGTTTGGTAAG |  |
| FCP  1536 | VC2744-P4 | CGCGAGCTCGCTGACCAATATTTGGCAG | SacI |
| FCP  1537 | VC2744-check | GCCGCCAATCATTATGTCTC |  |
| FCP  2437 | VC2744 check rev | TTGGCACATCAATACACTGA |  |
| FCP  1317 | VCA0952 P1 | GGATGTCGACGCTATTGATATTC | SalI | *vpsT* clean deletion mutant  (pCVD442 Δ*vpsT*) |
| FCP  1318 | VCA0952 P2 | TTTTTTGCGGCCGCTTTTTTCACATCAAGGCTAACATG |  |
| FCP  1319 | VCA0952 P3 | AAAAAAGCGGCCGCAAAAAAGTCAATTCTTAATTCGTTGTG |  |
| FCP  1320 | VCA0952 P4 | GGGTCGACTGCCGTAGTCGGTATC | SalI |
| FCP  1483 | VCA0952 check | CCAAGAATACATTCTCCATTATC |  |
| FCP  2434 | VCA0952 check rev | GGGTGGACGCTCTGGCACT |  |
| FCP  1307 | VC0665 P1 | GGGTCTAGATATTGGTATTGATCGTC | XbaI | *vpsR* clean deletion mutant  (pCVD442 Δ*vpsR*) |
| FCP  1308 | VC0665 P2 | TTTTTTGCGGCCGCTTTTTTCAGGGTTCATAGGTACCTC |  |
| FCP  1309 | VC0665 P3 | AAAAAAGCGGCCGCAAAAAACGCGTGCAACCATGTATC |  |
| FCP  1310 | VC0665 P4 | GCCATCTAGAGCCTCCAAGCAAAGTC | XbaI |
| FCP  1480 | VC0665 check | GGATGAGTCTCAGCTCGATC |  |
| FCP  2436 | VC0665 check rev | CGCACTTTACGCTTCCACACT |  |
| FCP  845 | *hapR* P1 | AAAATCTAGACTTCACGACGGGTTG | XbaI | *hapR* clean deletion mutant  (pCVD442 Δ*hapR*) |
| FCP  846 | *hapR* P2 | TTTTTTGCGGCCGCTTTTTTCATAGGGGTATATCCTTG |  |
| FCP  847 | *hapR* P3 | AAAAAAGCGGCCGCAAAAAAGTTTCTTGGGCAGCAC |  |
| FCP  848 | *hapR* P4 | AAAATCTAGACCATGTGCGGTGATC | XbaI |
| FCP  849 | *hapR* checkfw | GCACCATTACACTCATAGG |  |
| FCP  850 | *hapR* checkrv | CCTGCAACACCAAGTCG |  |
| FCP  845 | *hapR* P1 | AAAATCTAGACTTCACGACGGGTTG |  | Chromosomal Exchange by *hapR* from C6706  (pCVD442 *hapR*c) |
| FCP  848 | *hapR* P4 | AAAATCTAGACCATGTGCGGTGATC |  |
| FCP  2512 | VCA1078 P1 | CACGGTCTAGACAGGAACCTCTTGGTC | XbaI | *vqmA* clean deletion mutant  (pCVD442 Δ*vqmA*) |
| FCP  2517 | VCA1078 P2 | TTTTTTGCGGCCGCTTTTTTGGGGATCGTGTGATCGCTC |  |
| FCP  2518 | VCA1078 P3 | AAAAAAGCGGCCGCAAAAAAAGAGCAGATTTCTTTATTC |  |
| FCP  2515 | VCA1078 P4 | GCGATTCTAGATGCGACGGTAGATGAACAC | XbaI |
| FCP  2516 | VCA1078 check | GGTATGAGGCCAAACTACGCAG |  |
| FCP  3244 | VC1920 P1 | GGTTTGATtCtAGAGTTCATTGGTCGTCTG | XbaI | *lonA* clean deletion mutant  (pCVD442 Δ*lonA*) |
| FCP  3245 | VC1920 P2 | TTTTTTGCGGCCGCTTTTTTATTTTTCTCTCTTCCGCTTTG |  |
| FCP  3246 | VC1920 P3 | AAAAAAGCGGCCGCAAAAAAGCAAAAATAAGTAAATCTTTAC |  |
| FCP  3247 | VC1920 P4 | CCTCAGATCTAGAAATGCGCATCAGTAGGATG | XbaI |
| FCP  3248 | VC1920 check | GCGATGACGGTGGTGCGCTCTC |  |
| FCP  1204 | P*vpsR* HindIII fw | CCGAAGCTTACCAATACTCACACTATC | HindIII | P*vpsR*, translational β-galactosidase reporter |
| FCP  1206 | P*vpsR* EcoRI rev | CCATACGGGAATTCCCTTTCATGAACCTATATTC | EcoRI |
| FCP  1208 | P*vpsT* HindIII fw | GCATGAAGCTTAAGTGCTTTATCGCAC | HindIII | P*vpsT*, translational β-galactosidase reporter |
| FCP  1210 | P*vpsT* EcoRI rev | CGTTTGGAATTCCCTTTCATTTCACCCCTCCTAAC | EcoRI |
| FCP809 | P*vpsL* HindIII fw | GGCTAAGCTTCTTGTGTACATAGAGCAG | HindIII | P*vpsL*, translational β-galactosidase reporter |
| FCP5381 | P*vpsL* EcoRI rev | GTGCTTGAATTCCCTTTCATTAGACGCTCCTAAC | EcoRI |
| FCP5382 | P*vpsU* BglII fw | CCGCTCAGATCTAAAATTGGTTATCCCATC | BglII | P*vpsU*, translational β-galactosidase reporter |
| FCP5383 | P*vpsU* EcoRI rev | CCGAGAAAGAATTCCCTTTCATACCCTAACTAC | EcoRI |
| FCP779 | P*bap1* HindIII fw | GTCCGAAGCTTTGCTGAGTAAGACTC | HindIII | P*bap1*, translational β-galactosidase reporter |
| FCP5379 | P*bap1* EcoRI rev | CCACGTTGAATTCCCTTTCATGGCTTGACCTTC | EcoRI |
| FCP3025 | P*gyrA* HindIII fw | CCTTGAAGCTTTCTTGTATCGCCTGTG | HindIII | P*gyrA*, translational β-galactosidase reporter |
| FCP3026 | P*gyrA* EcoRI rev | CCTTAGAATTCCCTTTCATAGAGCCATTATCCCTC | EcoRI |
| FCP5388 | P*hfq* HindIII fw | GGAAGCAAAGCTTAATGTGATCGCTTTTGCGT | HindIII | P*hfq*, translational β-galactosidase reporter |
| FCP5389 | P*hfq* EcoRI rev | GTAGAGATGAATTCCCTTTCATTTTGTTTTTCCTTATTTTGTC | EcoRI |
| FCP5390 | P*mrcA* HindIII fw | CCGATTTAAGCTTGAGGCTAAGCTCCAG | HindIII | P*mrcA*, translational β-galactosidase reporter |
| FCP5391 | P*mrcA* EcoRI rev | CCCTGTTTGAATTCCCTTTCATTAATGCAAACCCTTGTAC | EcoRI |
| FCP  1687 | BamHI-RBS-VC2744 fw | GTGAAGGATCCAGGAGGACCCCGATGACCACTCCGCAAATTG | BamHI | Overexpression of *V.* *cholerae* co969 *bipA* from pHL100 |
| FCP  1688 | HindIII-VC2744 rev | CGAAAGTAAGCTTTTACTTCGCATCACGAGAAGC | HindIII |
| FCP  3101 | EcoRI-RBS-ECbipA fw | GCTATTTCTGAATTCAGGAGGAAAGTTGTGATCGAAAAATTG | EcoRI | Overexpression of *E.* *coli* MG1655 K-12 *bipA* from pHL100 |
| FCP  3102 | ECbipA-HindIII | GGGAAAAGCTTTTAATCGTCTTTCGGTG | HindIII |
| FCP  3103 | BamHI-RBS-PPbipA fw | GGATCCGGATCCAGGAGGACACTTGTGATCGAAAATCTG | BamHI | Overexpression of *P.* *putida* KT2440 *bipA* from pHL100 |
| FCP  3104 | PPbipA-HindIII rev | CCTGAaagcttTCAGTTCTTGGCTTTCTTG | HindIII |
| FCP  3249 | VC1920 SacI fw  (SacI-RBS-VC1920 fw) | GGTGCAAGAGCTCAGGAGGAAAAATATGAACTTGGAGCGTTC | SacI | Overexpression of *lonA* from pHL100 |
| FCP  3250 | VC1920 BamHI rev | CCACTTAGGATCCCTATGCATCACTCTTTTTGCTC | BamHI |
| FCP  2511 | NdeI-VC2744 fw | CAAACCCATATGACCACTCCGCAAATTG | NdeI | Purification of BipA-His (from pET22b-*bipA*-His) |
| FCP  2510 | VC2744-His-stop-HindIII-rev | GATGACTTAAGCTTTTAGTGGTGGTGGTGGTGGTGCTTCGCATCACGAGAAG | HindIII |
| FCP  1822 | VC2744-flag P1 | CGTCTTCTAGACCATTCATTTGACCACTAC | XbaI | *bipA*-flag chromosomal phusion |
| FCP  1823 | VC2744-flag P2 | TTACTTATCGTCATCGTCCTTGTAGTCCTTCGCATCACGAGAAGCAC |  |
| FCP  1824 | VC2744-flag P3 | GACTACAAGGACGATGACGATAAGTAATCACTGACTTTCGTTTG |  |
| FCP  1825 | VC2744-flag P4 | CCTGTTCTAGATAGCCCGAGCTCGCTGAC | XbaI |
| FCP  2437 | VC2744 check rev | TTGGCACATCAATACACTGA |  |
| FCP  1461 | flag fw | GACTACAAGGACGATGACGATAAG |  | Checking flag chromosomal fusions |
| FCP  718 | CVD442 F | CCAGCCCTCCTGTTTGAAGATG |  | Checking and sequencing inserts into pCVD442 |
| FCP  719 | CVD442 R | ACTGAGAAGCCCTTAGAGCC |  |
| FCP  224 | YPR505 | GCCGACATCATAACGGTTCTGG |  | Checking and sequencing inserts into pHL100 |
| FCP  643 | Pbad33-BKD | CAGGCTGAAAATCTTCTCTCATCC |  |
| FCP  39 | T7 forward | TAATACGACTCACTATAGGG |  | Checking and sequencing inserts into pET22b(+) |
| FCP  40 | T7 terminator primer | CTAGTTATTGCTCAGCGGTG |  |

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