Phenotype-guided comparative genomics identifies the complete transport pathway of the antimicrobial lasso peptide ubonodin in *Burkholderia*

Supporting Information

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ADDITIONAL MATERIALS AND METHODS

Safety statement

No unexpected or unusually high safety hazards were encountered in the course of this study.

Culture conditions

Unless otherwise indicated, all bacterial strains were cultured in Luria-Bertani Miller broth (10 g L^{-1} tryptone, 10 g L^{-1} NaCl, 5 g L^{-1} yeast extract). For spot-on-lawn assays, LB and M63 media (2 g L^{-1} $(NH_4)_2SO_4$, 13.6 g L^{-1} KH_2PO_4, 0.2% (w/v) D-glucose, 1 mM $MgSO_4$, 0.04 g L^{-1} each of the 20 standard amino acids, 0.00005% (w/v) thiamine) were used. For MIC liquid inhibition assays, cation-adjusted Mueller-Hinton II broth was also used (3 g L^{-1} beef extract, 17.5 g L^{-1} acid hydrolysate of casein, 1.5 g L^{-1} starch, 10 mg L^{-1} Mg^{2+}, 20 mg L^{-1} Ca^{2+}). *E. coli* strains were grown at 37 °C overnight. *B. cepacia* strain ATCC 25416 was typically grown at 30 °C for 1–2 days until distinct colonies appeared. All Bcc clinical isolates were grown at 32–35 °C for 1–3 days until colonies appeared. Antibiotics were used at the following concentration for selection in *E. coli*: 100 µg mL^{-1} ampicillin, 25 µg mL^{-1} chloramphenicol, 50 µg mL^{-1} trimethoprim, 50 µg mL^{-1} kanamycin, and 50 µg mL^{-1} gentamicin. For selection in *B. cepacia*, antibiotics were used at the following concentrations: 300 µg mL^{-1} kanamycin, 50–100 µg mL^{-1} trimethoprim, and 50 µg mL^{-1} chloramphenicol.

Strain construction

The list of strains used in this study can be found in **Table S9**. Chemical transformation was used to introduce plasmids into *E. coli* XL1-Blue Mix & Go competent cells (Zymo Research, cat. no. T3002) or *E. coli* DH5α chemically competent cells. Electroporation was used to introduce pir-dependent plasmids into electrocompetent *E. coli* CC118(λpir) and *E. coli* S17-1(λpir) cells. Electroporation was also used to introduce plasmids into electrocompetent *E. coli* BL21 and BL21(DE3) Δ*slyD* cells. Abbreviations used in this and the plasmid construction sections: Cm^R, chloramphenicol resistance; $Gent^R$, gentamicin resistance, Tp/TpTer/Tp^R, trimethoprim resistance; Km/Km^R, kanamycin resistance.

TD042 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_06959 (*tonB3*)::TpTer]. The donor strain *E. coli* S17-1(λpir) propagating the pTD005-[pSHAFT3-Δ*tonB3*::TpTer] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416. Tp^RGent^R transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD045-oTD046 and oTD045-oTD043.

TD075 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_00610 (*fur*)::Km; pSCrhaB2-*fur*]. This strain was made in two steps by first introducing the *fur* expression plasmid and then replacing chromosomal *fur* with a Km^R marker. The donor strain *E. coli* DH5α propagating pTD013-[pSCrhaB2-*fur*] and helper strain *E. coli* DH5α/pRK2013 were triparentally mated with recipient strain *B. cepacia* ATCC 25416 to transfer the expression plasmid. Tp^RGent^R transconjugant colonies were checked by colony PCR using primer pairs oTD035-oTD036 to confirm transfer of pTD013. Next, the donor strain *E. coli* S17-1(λpir) propagating the pTD016-[pTD014-Δ*fur*::Km] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416; pSCrhaB2-*fur*. Km^RGent^R transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD062-oTD063 and oTD063-oTD091. Strain TD075 needed to be propagated on LB selection plates supplemented with 0.02–0.2% (w/v) L-rhamnose as *fur* is essential.

TD078 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_07362 (*tonB4*)::Km]. The donor strain *E. coli* S17-1(λpir) propagating the pTD017-[pSHAFT3-Δ*tonB4*)::Km] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416. $Km^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD089-oTD090 and oTD090-oTD091.

TD080 [*B. cepacia* ATCC 25416; pSCrhaB2]. The donor strain *E. coli* DH5α propagating pSCrhaB2 and helper strain *E. coli* DH5α/pRK2013 were triparentally mated with recipient strain *B. cepacia* ATCC 25416 to transfer the empty vector. $Tp^R Gent^R$ transconjugant colonies were checked by colony PCR using primer pairs oTD035-oTD036 to confirm transfer of pSCrhaB2.

TD090 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_04892 (*tonB2*)::TpTer]. The donor strain *E. coli* S17-1(λpir) propagating the pTD022-[pTD014-Δ*tonB2*::TpTer] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416. $Tp^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD132-oTD133 and oTD132-oTD043.

TD091 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_02544 (*tonB1*)::Km; pSCrhaB2-*tonB1*]. This strain was made in two steps by first introducing the *tonB1* expression plasmid and then replacing chromosomal *tonB1* with a Km^R marker. The donor strain *E. coli* DH5α propagating pTD020-[pSCrhaB2-*tonB1*] and helper strain *E. coli* DH5α/pRK2013 were triparentally mated with recipient strain *B. cepacia* ATCC 25416 to transfer the expression plasmid. $Tp^R Gent^R$ transconjugant colonies were checked by colony PCR using primer pairs oTD035-oTD036 to confirm transfer of pTD020. Next, the donor strain *E. coli* S17-1(λpir) propagating the pTD019-[pTD014-Δ*tonB1*::Km] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416; pSCrhaB2-*tonB1*. $Km^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD118-oTD119 and oTD118-oTD091. Strain TD091 was propagated on LB selection plates supplemented with 0.02% (w/v) L-rhamnose as *tonB1* is important for healthy cell growth.

TD103 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_01381 (*pupB*)::Km]. The donor strain *E. coli* S17-1(λpir) propagating the pTD023-[pTD014-Δ*pupB*::Km] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416. $Km^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD151-oTD152.

TD118 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_01381 (*pupB*)::Km; pSCrhaB2-APZ15_10615 (*pupB*)]. This strain was made in two steps by first introducing the *pupB* expression plasmid and then replacing chromosomal *pupB* with a Km^R marker. The donor strain *E. coli* DH5α propagating pTD029-[pSCrhaB2-*pupB*] and helper strain *E. coli* DH5α/pRK2013 were triparentally mated with recipient strain *B. cepacia* ATCC 25416 to transfer the expression plasmid. $Tp^R Gent^R$ transconjugant colonies were checked by colony PCR using primer pairs oTD035-oTD036 to confirm transfer of pTD029. Next, the donor strain *E. coli* S17-1(λpir) propagating the pTD023-[pTD014-Δ*pupB*::Km] suicide plasmid was biparentally mated into recipient strain *B. cepacia* ATCC 25416; pSCrhaB2-*pupB*. $Km^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD151-oTD152.

TD122 [*B. cepacia* ATCC 25416 ΔGGFLHMPP_00373 (*yddA*)::TpTer]. The donor strain *E. coli* S17-1(λpir) propagating the pTD032-[pTD014-Δ*yddA*::TpTer] suicide plasmid was biparentally

mated into recipient strain *B. cepacia* ATCC 25416. $Tp^R Gent^R$ transconjugant colonies were screened to identify double-crossover mutants which are also Cm^S and further confirmed by colony PCR using primer pairs oTD180-oTD179 and oTD180-oTD043.

Plasmid construction

The list of plasmids and oligonucleotides used in this study can be found in **Tables S10** and **S11**, respectively. Plasmids were cloned using *E. coli* XL1-Blue and *E. coli* DH5α for general purposes, but pir-dependent pSHAFT3-based plasmids were cloned using *E. coli* CC118(λpir). The pWC99 plasmid for ubonodin purification was overexpressed in *E. coli* BL21. Plasmids for *B. cepacia* PupB and/or TonB1 overexpression were freshly transformed into *E. coli* BL21(DE3) Δ*slyD*. When needed, bacterial genomic DNA was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, cat. no. 69504) according to manufacturer's protocol. The Q5® High-Fidelity DNA Polymerase and buffers (NEB, cat. no. M0491L) were used for PCR. Other standard cloning reagents include T4 DNA ligase (NEB), restriction enzymes (NEB), Zymoclean Gel DNA Recovery Kits (Zymo Research), and QIAprep Spin Miniprep Kits (QIAGEN). All plasmids were sequenced using the GENEWIZ/Azenta Life Sciences sequencing service to confirm the correct insert was cloned.

pTD005 [pSHAFT3-ΔGGFLHMPP_06959 (*tonB3*)::TpTer]. The 692-bp sequence upstream of the *tonB3* ATG start codon was PCR-amplified using primer pairs oTD026-oTD027 and ATCC 25416 gDNA template. The PcS-TpTer cassette was PCR-amplified using primer pairs oTD029-oTD030 and p34E-TpTer plasmid template. The 783-bp sequence downstream of *tonB3* Ser227 codon was PCR-amplified using primer pairs oTD028-oTD025 and ATCC 25416 gDNA template. The 3 PCR products were stitched together with T4 DNA ligase and cloned between the EcoRI and XbaI sites of pSHAFT3.

pTD013 [pSCrhaB2-GGFLHMPP_00610 (*fur*)]. GGFLHMPP_00610 [Met1–His142] was PCR-amplified using primer pairs oTD083-oTD084 and ATCC 25416 gDNA template. The PCR product was cloned between the NdeI and BamHI sites of pSCrhaB2.

pTD014 [pSHAFT3-Amp^R Cyt717Ade-BsaI-PGphD-mCherry-BsaI]. This plasmid was made in two steps. First, the Amp^R [Ile140–Gly242] sequence was PCR-amplified using primer pairs oTD064-oTD065 and pSHAFT3 plasmid template. Primer oTD064 mutates out the native BsaI within the pSHAFT3 Amp^R sequence. The PCR product was cloned between the BsaI and PvuI sites of pSHAFT3. Next, the PGphD promoter was PCR-amplified using primer pairs oTD076-oTD078 and pYTK001 plasmid template, a plasmid made available on Addgene (#65108) from John Dueber[1]. The mCherry sequence was PCR-amplified using primer pairs oTD079-oTD080 and pET28a-mCherry-CNA35 plasmid template, a plasmid made available on Addgene (#61607) from Maarten Merkx[2]. Overlap PCR was performed to stitch together the PGphD and mCherry products, and the resultant product was cloned between the EcoRI and XbaI sites of the plasmid generated in the first step.

pTD016 [pTD014-ΔGGFLHMPP_00610 (*fur*)::Km]. The 702-bp sequence upstream of the *fur* Thr5 codon was PCR-amplified using primer pairs oTD094-oTD095 and ATCC 25416 gDNA template. The PcS-Km cassette was PCR-amplified using primer pairs oTD098-oTD099 and p34E-Km plasmid template. The 671-bp sequence downstream of the *fur* Arg140 codon was PCR-amplified using primer pairs oTD096-oTD097 and ATCC 25416 gDNA template. The 3 PCR products were cloned between the EcoRI and XbaI sites of pTD014 via Golden Gate cloning.

pTD017 [pSHAFT3-ΔGGFLHMPP_07362 (*tonB4*)::Km]. The 742-bp sequence upstream of the *tonB4* Met1 start codon was PCR-amplified using primer pairs oTD071-oTD104 and ATCC 25416

gDNA template. The PcS-Km cassette was PCR-amplified using primer pairs oTD106-oTD107 and p34E-Km plasmid template. The 716-bp sequence downstream of the *tonB4* Phe223 codon was PCR-amplified using primer pairs oTD105-oTD068 and ATCC 25416 gDNA template. The 3 PCR products were stitched together using overlap PCR and cloned between the EcoRI and XbaI sites of pSHAFT3.

pTD019 [pTD014-ΔGGFLHMPP_02544 (*tonB1*)::Km]. The 615-bp sequence upstream of the *tonB1* Pro3 codon was PCR-amplified using primer pairs oTD114-oTD115 and ATCC 25416 gDNA template. The PcS-Km cassette was PCR-amplified using primer pairs oTD098-oTD099 and p34E-Km plasmid template. The 588-bp sequence downstream of the *tonB1* stop codon was PCR-amplified using primer pairs oTD116-oTD117 and ATCC 25416 gDNA template. The 3 PCR products were cloned between the EcoRI and XbaI sites of pTD014 via Golden Gate cloning.

pTD020 [pSCrhaB2-GGFLHMPP_02544 (*tonB1*)]. GGFLHMPP_02544 [Met1–Asp226] was PCR-amplified using primer pairs oTD120-oTD121 and ATCC 25416 gDNA template. The PCR product was cloned between the NdeI and BamHI sites of pSCrhaB2.

pTD022 [pTD014-ΔGGFLHMPP_04892 (*tonB2*)::TpTer]. The 670-bp sequence upstream of the *tonB2* Met1 codon was PCR-amplified using primer pairs oTD128-oTD129 and ATCC 25416 gDNA template. The PcS-TpTer cassette was PCR-amplified using primer pairs oTD029-oTD030 and p34E-TpTer plasmid template. The 625-bp sequence downstream of the *tonB2* Asp269 codon was PCR-amplified using primer pairs oTD130-oTD131 and ATCC 25416 gDNA template. The 3 PCR products were cloned between the EcoRI and XbaI sites of pTD014 via Golden Gate cloning.

pTD023 [pTD014-ΔGGFLHMPP_01381 (*pupB*)::Km]. The 686-bp sequence upstream of the *pupB* Val2 codon was PCR-amplified using primer pairs oTD134-oTD135 and ATCC 25416 gDNA template. The PcS-Km cassette was PCR-amplified using primer pairs oTD098-oTD099 and p34E-Km plasmid template. The 724-bp sequence downstream of the *pupB* Leu840 codon was PCR-amplified using primer pairs oTD136-oTD137 and ATCC 25416 gDNA template. The 3 PCR products were cloned between the EcoRI and XbaI sites of pTD014 via Golden Gate cloning.

pTD027 [pRSFDuet-1-GGFLHMPP_02544 (*tonB1*)]. GGFLHMPP_02544 [Met1–Asp226] was PCR-amplified using primer pairs oTD168-oTD169 and ATCC 25416 gDNA template. The PCR product was cloned between the NcoI and HindIII sites of pRSFDuet-1.

pTD029 [pSCrhaB2-APZ15_10615 (*pupB*)]. APZ15_10615 [Met1–Phe858] was PCR-amplified using primer pairs oTD173-oTD174 and ATCC 25416 gDNA template. The PCR product was cloned between the NdeI and HindIII sites of pSCrhaB2.

pTD030 [pRSFDuet-1-GGFLHMPP_02544 (*tonB1*)-APZ15_10615 (*pupB*)]. APZ15_10615 [Met1–Phe858] was PCR-amplified using primer pairs oTD173-oTD170 and ATCC 25416 gDNA template. The PCR product was cloned between the NdeI and AvrII sites of pTD027.

pTD031 [pRSFDuet-1-APZ15_10615 (*pupB*)]. APZ15_10615 [Met1–Phe858] was PCR-amplified using primer pairs oTD173-oTD170 and ATCC 25416 gDNA template. The PCR product was cloned between the NdeI and AvrII sites of pRSFDuet-1.

pTD032 [pTD014-ΔGGFLHMPP_00373 (*yddA*)::TpTer]. The 634-bp sequence upstream of the *yddA* Met1 codon was PCR-amplified using primer pairs oTD175-oTD176 and ATCC 25416 gDNA template. The PcS-TpTer cassette was PCR-amplified using primer pairs oTD029-oTD030

and p34E-TpTer plasmid template. The 605-bp sequence downstream of the *yddA* Val581 codon was PCR-amplified using primer pairs oTD177-oTD178 and ATCC 25416 gDNA template. The 3 PCR products were cloned between the EcoRI and XbaI sites of pTD014 via Golden Gate cloning.

Biparental mating

Biparental mating was used to transfer pSHAFT3-based suicide plasmids[3] from *E. coli* into *B. cepacia* for allelic replacement. Briefly, 50 µL each of overnight cultures of the donor strain propagating the suicide plasmid and the recipient strain were mixed. The cell mixture was centrifuged at 6000 xg for 2 min at room temperature, and the cell pellet was resuspended with 50 µL of LB and spotted onto an LB agar plate without antibiotic selection. The plate was incubated face up at 30 °C overnight. The next day, the mating spot was scraped up with an inoculation loop and resuspended in 200 µL of sterile 1X PBS buffer. The resuspended cell mixture was plated on the appropriate selection plates to select for transconjugants. Gentamicin was used to select against the donor strain. Transconjugant colonies were picked and patched to screen for Cm^S indicating double-crossover mutants that have lost the wild-type allele.

Triparental mating

Triparental mating was used to transfer pSCrhaB2-based plasmids from *E. coli* into *B. cepacia*. Our study adapted a previously published protocol for triparental mating[4]. Briefly, 50 µL each of overnight cultures of the donor strain propagating the cargo plasmid, the helper strain *E. coli* DH5α/pRK2013[5], and the recipient strain were mixed. The cell mixture was centrifuged at 6000 xg for 2 min at room temperature, and the cell pellet was resuspended with 50 µL of LB and spotted onto an LB agar plate without antibiotic selection. The plate was incubated face up at 30 °C overnight. The next day, the mating spot was scraped up with an inoculation loop and resuspended in 200 µL of sterile 1X PBS buffer. The resuspended cell mixture was plated on the appropriate selection plates to select for transconjugants. Gentamicin was used to select against the donor strain.

Expression and purification of ubonodin

Ubonodin was overexpressed and purified as previously reported[6]. Briefly, BL21 propagating pWC99 was grown in M9 media (6.4 mg L^{-1} Na_2HPO_4 • 7 H_2O, 1.5 mg L^{-1} KH_2PO_4, 0.5 mg L^{-1} NH_4Cl, 0.25 mg L^{-1} NaCl, 0.15 µg mL^{-1} $CaCl_2$, 1 mM $MgSO_4$, 0.2% (w/v) D-glucose, 0.04 g L^{-1} each of the 20 standard amino acids, 0.00005% (w/v) thiamine) and ubonodin expression was induced by the addition of 1 mM IPTG. Cultures were grown for ~20 h at 20 °C following induction and then centrifuged to harvest the supernatant. The supernatant was extracted through a Strata C8 column, eluted with 100% methanol, and dried by rotovapping. The dried extract was resuspended with 25% acetonitrile/75% water and further purified by semi-preparative reverse-phase HPLC using an Agilent Technologies 1200 series instrument fitted with a Zorbax 300SB-C18 column (9.4 mm x 250 mm, 5 µm). Purified ubonodin was lyophilized and resuspended in water. The purity was checked by LC-MS and the concentration was measured using the A280 Nanodrop ND-1000 Spectrophotometer reading.

Cladogram construction

To construct the cladogram of Bcc strains tested for ubonodin susceptibility, first the allelic profile for 7 Bcc housekeeping genes (*atpD*, *gltB*, *gyrB*, *recA*, *lepA*, *phaC*, and *trpB*) and sequence type of each strain were retrieved from the *Burkholderia* Genome Database[7]. Then, the allelic profile and sequence type were entered into the PubMLST website (Public databases for molecular

typing and microbial genome diversity) to obtain the concatenated sequences of fragments of the 7 genes listed above. Finally, the concatenated sequences for all Bcc strains were aligned using Clustal Omega to generate a cladogram[8].

RNA polymerase protein sequence alignment

To generate a percent sequence identity matrix comparing the RNA polymerase β and β' subunits encoded by the Bcc strains tested for ubonodin susceptibility, first the RpoB and RpoC sequences for each strain were retrieved from NCBI. The concatenated RpoBC sequences were then aligned using Clustal Omega[8].

Spot dilution assay

To assess cell viability by spot dilution assay, overnight cultures of test strains were diluted 1:100 into fresh LB with antibiotics as needed the following morning and grown at 30 °C with shaking to an OD_{600} of ~0.4–0.6. Once the cultures reached mid-exponential phase, all cultures for the set of strains being tested were normalized to the OD_{600} of the least dense culture using LB. From this 10^0 starting dilution, ten-fold serial dilutions were prepared using LB. Five µL of each dilution was spotted onto the test media. The spots were left to dry in the biosafety cabinet with the lid slightly ajar. The plates were incubated ~15 h at the appropriate temperature and imaged the next day using the Bio Rad ChemiDoc XRS Gel Imaging System under epi white illumination and the Quantity One 4.6.6 imaging software. Images were processed using FIJI. Spot dilution assays were performed with at least 2 biological replicates (independent cultures) for each tested strain.

Liquid inhibition assay and bacterial growth rate analysis

To assess the endpoint MIC of ubonodin by liquid inhibition assay, overnight cultures of test strains were diluted 1:100 into fresh LB with antibiotics as needed and grown at 30 °C with shaking to an OD_{600} of ~0.4–0.6. Once the cultures reached mid-exponential phase, they were diluted to an OD_{600} of 0.001 in the test media. Two-fold serial dilutions of ubonodin (0–32 µM) were prepared in test media and 50 µL of each dilution was dispensed into an untreated, sterile 96-well plate. The ubonodin dilutions were topped off with an equivalent volume (50 µL) of the 0.001 OD_{600} cells, bringing the final OD_{600} to 0.0005 in each well and the range of ubonodin concentrations to 0–16 µM. The 96-well plate was covered with a matching lid and incubated with shaking at 30 °C for ~16 h. The endpoint OD_{600} was measured using a BioTek Synergy 4 plate reader and blanked to a test media only control to calculate the MIC of ubonodin. When needed, the test medium was supplemented with L-rhamnose to induce protein expression. Both cation-adjusted Mueller-Hinton Broth II and LB were used as test media for liquid inhibition assays; the media used in a certain assay is indicated within the corresponding figure caption.

To assess bacterial growth over time, exponential-phase LB cultures of test strains inoculated from a scoop of freshly-streaked colonies were diluted to a final OD_{600} of 0.001 in 150 µL of test media in a 96-well plate. The plate was covered with a matching lid and incubated with shaking at 30 °C in a BioTek Synergy 4 plate reader. OD_{600} measurements were recorded at 20-min intervals for 20–24 h.

Ubonodin cellular uptake assay

The endpoint concentration of ubonodin in the cell pellet was analyzed as a measure of cellular uptake. Overnight cultures of test strains were diluted 1:100 into fresh LB and grown at 30 °C with shaking to an OD_{600} of ~0.4–0.6. The cultures were diluted to an OD_{600} of 0.01 in M63 broth and

3 x 150 µL aliquots were dispensed into separate wells of an untreated, sterile 96-well plate. One well was left untreated, the second well contained 1 µM ubonodin, and the third well contained 2 µM ubonodin. The plate was covered with a matching lid and incubated with shaking at 30 °C for ~16 h. The endpoint OD_{600} was measured using a BioTek Synergy 4 plate reader and blanked to an M63 media only control. The content of each well was transferred to a 1.5-mL microcentrifuge tube and the cell pellet was harvested at 6000 xg for 2 min at room temperature. The supernatant was removed and the cell pellet was washed once with 200 µL of sterile 1X PBS before a second spin to remove the wash fraction.

We adapted a previously published bacterial cell lysis protocol to release intracellular metabolites for LC-MS analysis[9]. The cell pellet was resuspended in 200 µL of cold 100% methanol. The cell resuspension was transferred to dry ice for 5 min, followed by thawing at room temperature and vortexing for 30 sec. This freeze-thaw-vortex step was repeated 3 times in total and the lysate was spun down at 10000 xg for 5 min at room temperature to harvest the supernatant and pellet out the cell debris. A second extraction step was performed to maximize cell lysis. The cell debris pellet was resuspended with another 200 µL of cold 100% methanol and subjected to another round of freeze-thaw-vortex before centrifugation. The supernatant from this second extraction was combined with the first extraction. To evaporate off the methanol of all samples in parallel, the methanol extracts were left in the fume hood at room temperature for ~24 h with the cap open.

The dried extracts were resuspended with 75 µL of sterile ultrapure water and centrifuged at 10000 xg for 15 min at room temperature to pellet out debris. Thirty-five µL of the supernatant was transferred to an LC-MS vial fitted with a sample insert and 25 µL was injected onto the LC-MS for analysis. LC-MS analysis was performed using a Zorbax 300SB-C18 column (2.1 mm x 50 mm, 3.5 µm) installed on an Agilent 1260 Infinity II system in line with an Agilent 6530 Q-TOF mass spectrometer. The LC-MS gradient used for separation was: 0.5 mL min^{-1} 90% solvent A (H_2O, 0.1% formic acid) and 10% solvent B (acetonitrile, 0.1% formic acid) for 1 min followed by a linear gradient to 50% solvent B over 19 min, then a linear gradient to 90% solvent B over 5 min, and a linear gradient back to 10% solvent B over 5 min. As ubonodin undergoes oxidation[6] especially upon air drying in the fume hood, multiply-oxidized ubonodin species were observed by LC-MS. To calculate the intracellular concentration of ubonodin for each sample, the sum of the extracted ion counts of non-oxidized ubonodin and the ubonodin +16/32/48/64 species was calculated for the total sample volume of 75 µL and normalized to the final OD_{600}.

Computational prediction of Fur DNA-binding sites

To predict Fur DNA-binding sites in the *B. cepacia* ATCC 25416 genome, the custom Python script PWMmodel.py was implemented on the Princeton Della server. This analysis was adapted from bioinformatics methods used for identification of regulatory elements[10]. It first required a set of known Fur DNA-binding site sequences, which are in general well-conserved. Seven experimentally-determined 19-bp Fur binding sequences from *P. aeruginosa* were used as the known set[11–14]. Alignment of these 7 sequences using the online MEME tool[15] generated a sequence logo summarizing the Fur consensus sequence. From these 7 sequences, a position frequency matrix (PFM) was also generated by calculating the number of times each nucleotide (A, C, G, T) was found at each position within the 19-bp sequence. The PFM captures the nucleotide characteristics at each position. The PFM was then converted to a position weight matrix (PWM) by calculating the probability of observing a particular nucleotide at a specific position normalized to the expected background probability. The *B. cepacia* ATCC 25416 genome is ~67% GC-rich, so 16.5% was expected for A/T each and 33.5% was expected for G/C each as the background probabilities. Using the PWM, any 19-bp sequence can be scored for how similar it is to the expected Fur binding site sequence. This is done by summing the individual PWM

scores corresponding to an observed nucleotide at each consecutive position. The PWM built from the 7 known Fur binding sites was used to scan the ATCC 25416 genome for high-scoring Fur boxes. The scan made use of a 19-bp sliding window, which shifted by 1 nucleotide in each iteration, on both the forward and reverse strands. As many genome fragments are analyzed and most produce low match scores, this study filtered for Fur box hits that are found proximal to, but not within, and facing the same direction as a coding DNA sequence (CDS). It also filtered for Fur box hits in terms of information content, a measure of conservation at each position. To generate histograms of the match scores for all or a subset of scanned fragments, the custom Python script PWM-annotator.py was implemented on the Della server. All custom scripts used in this analysis can be found on the Link lab Github page.

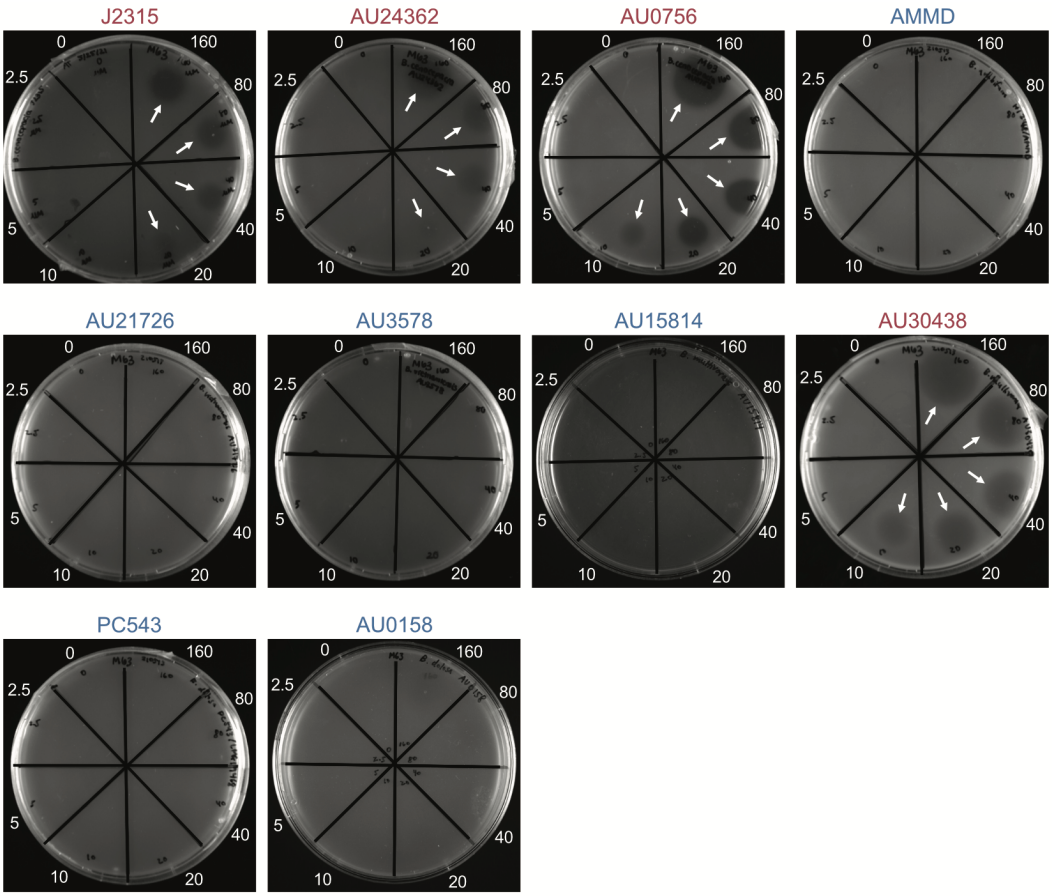

Figure S1. Spot-on-lawn assay assessing Bcc strains for ubonodin susceptibility. Cultures were grown to exponential phase, mixed with M63 soft agar, and plated on M63 base agar. Two-fold serial dilutions of ubonodin ranging from 0–160 μM were spotted onto the respective sectors. For select strains, clear zones (arrows) indicating bacterial growth inhibition were observed. Ubonodin-susceptible strains are denoted in red and non-susceptible strains in blue. We note that *B. multivorans* AU15814 grew poorly on M63 compared to the other Bcc strains, which formed a lawn of cells.

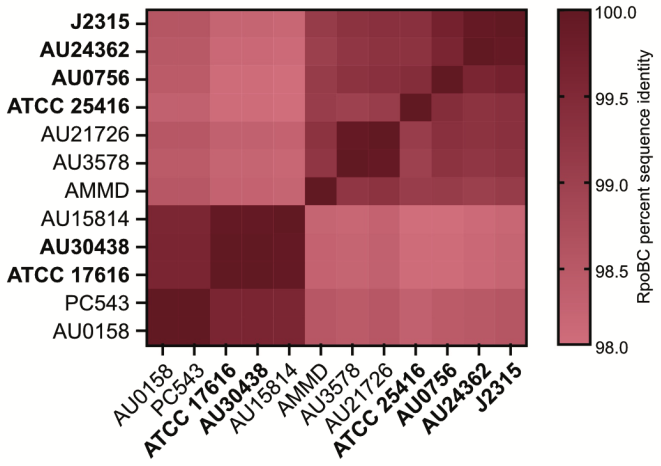

Figure S2. Conservation of the RNA polymerase β and β' subunits across the Bcc strains tested for ubonodin susceptibility. Heat map showing percent sequence identity when the concatenated amino acid sequences of RpoB (β subunit) and RpoC (β' subunit) were aligned with Clustal Omega. A darker shade represents more identical RpoBC sequences for a pair of strains along the x- and y-axes. Ubonodin-susceptible Bcc strains are bolded.

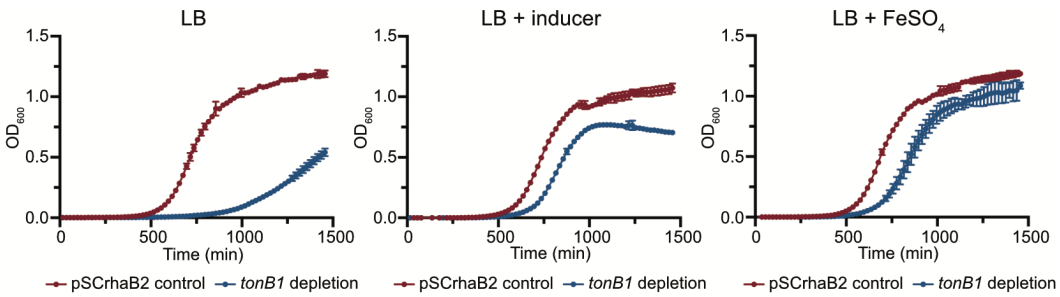

Figure S3. Iron supplementation mitigates the defect in *B. cepacia* cell growth due to *tonB1* depletion. The *tonB1* depletion mutant and empty vector control strain were grown in LB (left graph) and in the presence of 0.02% L-rhamnose (middle graph) or 200 μM $FeSO_4$ (right graph). The mean ± standard deviation for technical triplicates from the same overnight culture is shown.

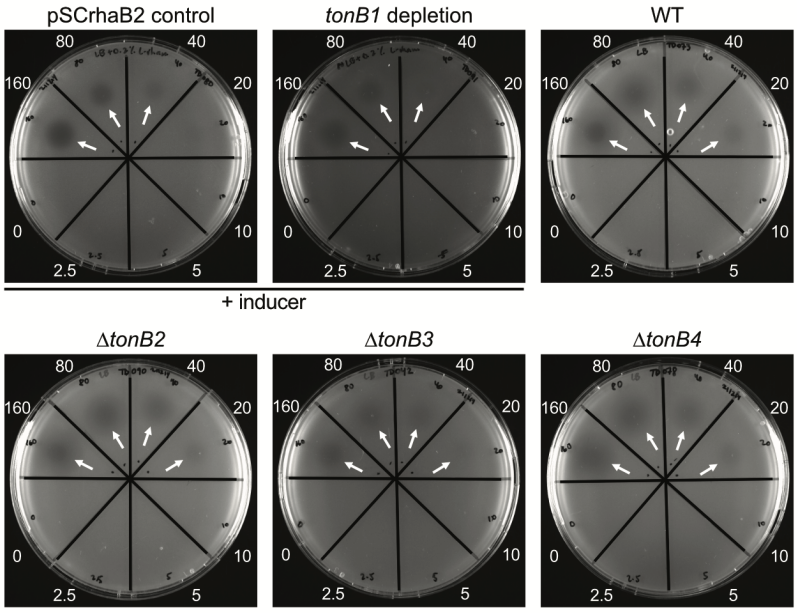

Figure S4. A single TonB pathway is required for ubonodin activity. Spot-on-lawn LB plates with the *tonB1* depletion and empty vector control strains, *B. cepacia* WT, and deletion mutants of the other 3 *tonB* homologs were examined for susceptibility to 0–160 μM of ubonodin. Ubonodin inhibition was observed (arrows) when *tonB1* was expressed (+ 0.2% L-rhamnose) and loss of the other TonBs did not impact the MIC.

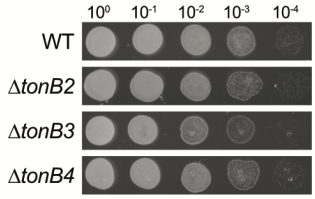

Figure S5. Most TonB homologs are non-essential in *B. cepacia*. Ten-fold serial dilutions of cultures were spotted onto LB agar.

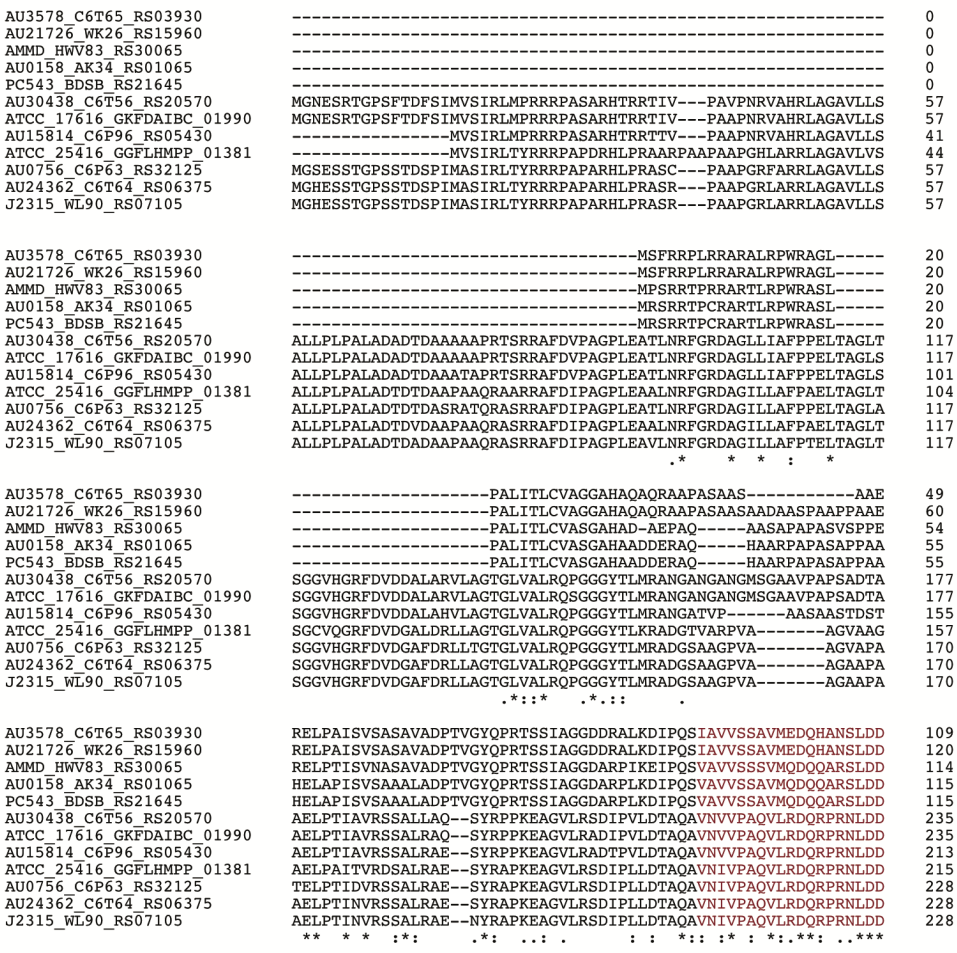

Figure S6. PupB homologs encoded by ubonodin-susceptible strains harbor an N-terminal extension domain. The closest PupB homologs found by protein BLAST search against the 12 Bcc strains tested for ubonodin susceptibility were aligned with Clustal Omega. Only the N-terminal portion of the alignment is shown here. The start of the plug domain based on protein BLAST annotation of the ATCC 25416 PupB homolog is denoted in red text.

Strain	Ubonodin MIC (μM)
Burkholderia multivorans AU30438	10
Burkholderia multivorans ATCC 17616	20
Burkholderia cepacia ATCC 25416	40
Burkholderia cenocepacia AU0756	10
Burkholderia cenocepacia AU24362	20
Burkholderia cenocepacia J2315	20

Table S1. MICs of ubonodin against susceptible Bcc strains. Minimum inhibitory concentration measurements were obtained via M63 spot-on-lawn assay and observing for the lowest ubonodin concentration at which a zone of inhibition was clearly visible. MIC values for strains ATCC 17616 and ATCC 25416 were previously reported[6].

Predicted TBDT genes	Annotated function	*Ec*K-12 pident
GGFLHMPP_00194	Ferrichrome receptor FcuA	21.29
GGFLHMPP_00997	Vitamin B12 transporter BtuB	17.17
GGFLHMPP_01354	Ferrichrome outer membrane transporter/phage receptor	23.90
GGFLHMPP_01381	Ferric-pseudobactin BN7/BN8 receptor	24.74
GGFLHMPP_01830	Ferrichrome outer membrane transporter/phage receptor	34.04
GGFLHMPP_01957	Vitamin B12 transporter BtuB	17.21
GGFLHMPP_03614	Fe(3+)-pyochelin receptor	23.10
GGFLHMPP_03754	putative TonB-dependent receptor BfrD	23.29
GGFLHMPP_03764	Vitamin B12 transporter BtuB	15.48
GGFLHMPP_03765	Ferrichrome outer membrane transporter/phage receptor	21.79
GGFLHMPP_04498	Vitamin B12 transporter BtuB	20.28
GGFLHMPP_04684	Ferrichrome outer membrane transporter/phage receptor	25.16
GGFLHMPP_04786	Vitamin B12 transporter BtuB	16.18
GGFLHMPP_04897	Vitamin B12 transporter BtuB	17.68
GGFLHMPP_05058	Ferric-anguibactin receptor FatA	22.47
GGFLHMPP_05298	Vitamin B12 transporter BtuB	15.37
GGFLHMPP_05484	putative TonB-dependent receptor BfrD	21.47
GGFLHMPP_05932	putative TonB-dependent receptor	16.03
GGFLHMPP_05969	Vitamin B12 transporter BtuB	19.90
GGFLHMPP_06227	Fe(3+)-pyochelin receptor	24.02
GGFLHMPP_06471	putative TonB-dependent receptor BfrD	20.55
GGFLHMPP_06887	Ferrichrome outer membrane transporter/phage receptor	23.88
GGFLHMPP_06963	Colicin I receptor	14.80
GGFLHMPP_06967	Vitamin B12 transporter BtuB	16.72
GGFLHMPP_07045	Ferrichrome outer membrane transporter/phage receptor	32.24
GGFLHMPP_07356	Vitamin B12 transporter BtuB	16.83
GGFLHMPP_07363	Vitamin B12 transporter BtuB	13.21
GGFLHMPP_07399	Pesticin receptor	17.02
GGFLHMPP_07465	Ferrichrome outer membrane transporter/phage receptor	37.03

Table S2. Protein BLAST prediction of TonB-dependent transporters (TBDTs) encoded by *B. cepacia*. *E. coli* FhuA and 34 known and predicted *P. aeruginosa* TBDTs were queried against a protein BLAST database of ATCC 25416 built using the BLAST+ software package[16]. Only protein hits with an e-value < 0.05 and appearing as hits for multiple queries are shown here. For each TBDT homolog, the percent amino acid sequence identity (pident) to *E. coli* K-12 FhuA was calculated using Clustal Omega.

Predicted TonB genes	Annotated function	*Bc*K56-2 pident	*Ec*K-12 pident
GGFLHMPP_02544 (*tonB1*)	TonB	79.91	27.94
GGFLHMPP_04892 (*tonB2*)	TonB	33.65	28.24
GGFLHMPP_06959 (*tonB3*)	TonB	28.50	33.84
GGFLHMPP_07362 (*tonB4*)	TonB	30.69	32.02

Table S3. Protein BLAST prediction of TonB homologs in *B. cepacia*. Confirmed TonB sequences from *B. cenocepacia* K56-2[17] and *B. mallei* ATCC 23344[18] were queried against the same protein BLAST database of ATCC 25416 as above. Only top hits having a single Phobius-predicted transmembrane domain are shown here. For each TonB homolog, the percent amino acid sequence identity (pident) to TonB from *B. cenocepacia* K56-2 and *E. coli* K-12 was calculated using Clustal Omega.

Strain	- inducer, MIC (μM)	+ inducer, MIC (μM)
pSCrhaB2 control	40	40
tonB1 depletion	> 160	40
WT	20	na
Δ*tonB2*	20	na
Δ*tonB3*	20	na
Δ*tonB4*	20	na

Table S4. MICs of ubonodin for *B. cepacia tonB* mutants. MIC was defined as the lowest concentration of ubonodin required to inhibit bacterial growth via spot-on-lawn assay. The *tonB1* depletion mutant was compared to the pSCrhaB2 empty vector control strain on LB agar ± the inducer (0.2% L-rhamnose). For the *tonB2*, *tonB3*, and *tonB4* mutants for which the gene could be deleted, MICs were assessed against *B. cepacia* WT on plain LB agar. na indicates not applicable.

Gene	Log_2 fold change	Adjusted p-value	Annotated function
APZ15_13295	-5.2567923	7.81E-205	hemolysin D
APZ15_13300	-5.1862897	7.65E-202	multidrug resistance protein B
APZ15_13330	-4.9449433	1.84E-157	hypothetical protein
APZ15_13325	-4.7638721	6.55E-153	cystathionine beta-lyase
APZ15_13290	-4.8560812	5.49E-136	multidrug RND transporter
APZ15_13320	-4.7638147	2.45E-132	hypothetical protein
APZ15_13265	-4.4817844	1.38E-127	hypothetical protein
APZ15_13335	-4.5654781	1.84E-125	citrate lyase subunit beta
APZ15_13255	-3.8753646	2.07E-124	3-oxoacyl-ACP reductase
APZ15_13260	-4.4007282	1.49E-112	hypothetical protein
APZ15_13310	-4.5269245	1.84E-108	hypothetical protein
APZ15_13340	-4.4242902	5.51E-108	acyl-CoA dehydrogenase
APZ15_12790	-3.325007	1.32E-99	TonB-dependent receptor
APZ15_12770	-3.4803646	2.29E-99	non-ribosomal peptide synthetase
APZ15_13270	-4.2575782	3.25E-97	thioesterase
APZ15_12745	-3.4658715	1.24E-81	cobalamin/Fe3+-siderophore ABC transporter
APZ15_13315	-4.1908886	1.05E-79	glutamine amidotransferase
APZ15_12740	-3.4299677	2.04E-73	hypothetical protein
APZ15_12795	-2.9688796	2.19E-71	N(5)-hydroxyornithine transformylase PvdF
APZ15_13280	-3.2718661	1.60E-67	glutamate--cysteine ligase
APZ15_12775	-2.7586369	2.13E-65	non-ribosomal peptide synthetase
APZ15_13275	-3.1975719	1.75E-62	phospho-2-dehydro-3-deoxyheptonate aldolase
APZ15_12765	-2.8563236	6.22E-56	peptide ABC transporter ATP-binding protein
APZ15_12760	-2.6778373	5.44E-53	iron ABC transporter substrate-binding protein
APZ15_12785	-2.8072399	2.49E-52	ornithine monooxygenase
APZ15_12750	-2.9528878	1.09E-42	iron ABC transporter
APZ15_18790	-2.1337118	3.95E-42	ligand-gated channel protein
APZ15_28835	-2.2263542	4.46E-37	TonB-dependent receptor
APZ15_12755	-2.743308	1.02E-34	iron reductase
APZ15_12735	-3.0263176	3.41E-32	antibiotic synthesis protein MbtH
APZ15_16315	-1.6894186	1.16E-28	biopolymer transporter
APZ15_12800	-2.0448781	1.90E-24	acetyl-CoA acetyltransferase
APZ15_28830	-2.0801325	2.35E-21	hypothetical protein
APZ15_28825	-1.9648461	8.26E-21	Fe(II)-dependent oxygenase
APZ15_12730	-2.2044701	6.01E-19	RNA polymerase subunit sigma-70
APZ15_12780	-2.1739261	9.44E-19	ornibactin biosynthesis protein
APZ15_16320	-1.5356345	2.04E-18	biopolymer transporter ExbD
APZ15_13305	-2.352082	7.60E-16	hypothetical protein
APZ15_16305	-1.436329	3.05E-13	(2Fe-2S)-binding protein
APZ15_03205	-1.2202139	2.80E-12	peptidase
APZ15_09755	-1.1039095	3.75E-12	iron ABC transporter substrate-binding protein
APZ15_27265	-1.2298424	6.27E-12	TonB-dependent receptor
APZ15_09745	-1.0976609	1.90E-11	ABC transporter
APZ15_09750	-1.072656	6.92E-11	iron ABC transporter permease
APZ15_04725	-1.0998786	1.41E-10	acetyltransferase
APZ15_16350	-1.0260216	9.36E-10	FTR1 family iron permease
APZ15_16630	-0.9849508	1.68E-09	hypothetical protein
APZ15_22715	-1.1368288	9.35E-09	TonB-dependent receptor
APZ15_27905	-0.9797576	2.69E-08	AraC family transcriptional regulator
APZ15_16345	-0.939572	1.20E-07	hypothetical protein
APZ15_04730	-0.9347659	2.08E-07	hypothetical protein
APZ15_16355	-1.093043	3.08E-07	periplasmic lipoprotein involved in iron transport
APZ15_16310	-0.9727399	6.01E-07	energy transducer TonB
APZ15_04900	-0.8889021	1.18E-06	flagellar biosynthetic protein FlhB
APZ15_10610	-1.2837685	1.75E-06	iron dicitrate transport regulator FecR
APZ15_16360	-0.9521986	1.99E-06	hypothetical protein
APZ15_23635	-1.2306567	2.27E-06	heme ABC transporter
APZ15_04150	-0.897314	7.25E-06	flagellar basal body protein FliL
APZ15_18795	-1.1516382	8.61E-06	phosphonoacetaldehyde dehydrogenase
APZ15_03255	-0.7594545	1.10E-05	flagellar export protein FliJ
APZ15_16625	-0.9256496	1.13E-05	carbonate dehydratase
APZ15_32030	-1.0078194	1.14E-05	Crp/Fnr family transcriptional regulator
APZ15_34675	-0.9235059	1.35E-05	hypothetical protein
APZ15_18770	-0.7711676	1.47E-05	L-asparagine permease
APZ15_32780	-1.1403315	1.94E-05	hypothetical protein
APZ15_11800	-1.0160764	1.95E-05	hypothetical protein
APZ15_04905	-0.7522172	2.80E-05	flagellar biosynthesis protein FlhA
APZ15_32675	-0.929239	4.76E-05	acetoacetyl-CoA reductase

Gene	Log_2 fold change	Adjusted p-value	Annotated function
APZ15_13250	-0.8369174	4.76E-05	LysR family transcriptional regulator
APZ15_32715	-0.9749203	5.95E-05	Crp/Fnr family transcriptional regulator
APZ15_04920	-0.6763806	6.84E-05	RNA polymerase subunit sigma-70
APZ15_04145	-0.7111133	7.00E-05	flagellar motor switch protein FliM
APZ15_17665	-0.7959913	7.31E-05	porin
APZ15_02535	-0.6960766	7.31E-05	adenosylmethionine-8-amino-7-oxononanoate aminotransferase
APZ15_11910	-0.9078761	8.70E-05	hypothetical protein
APZ15_11900	-1.0983907	0.00010956	hypothetical protein
APZ15_18775	-0.6690793	0.00011443	aspartate ammonia-lyase
APZ15_11935	-0.9221637	0.0001207	hypothetical protein
APZ15_04745	-0.6528012	0.0001207	hypothetical protein
APZ15_15590	-0.974034	0.00012489	elements of external origin
APZ15_32765	-1.0324918	0.00015241	heat-shock protein Hsp20
APZ15_23630	-1.0662754	0.00021923	hypothetical protein
APZ15_24725	-0.8031059	0.00021923	disulfide bond formation protein DsbA
APZ15_32735	-0.8834857	0.00024137	hypothetical protein
APZ15_30295	-0.6488224	0.00025835	magnesium-translocating P-type ATPase
APZ15_12685	-0.7372566	0.00027478	multidrug transporter
APZ15_29005	-0.7067352	0.00028188	aldehyde dehydrogenase
APZ15_32025	-0.8622411	0.00033077	two-component system response regulator
APZ15_11950	-0.8733183	0.00040075	hypothetical protein
APZ15_32040	-0.7601469	0.00042828	UDP-glucose 4-epimerase
APZ15_11940	-0.855181	0.00043772	hypothetical protein
APZ15_11945	-0.8255062	0.00049894	hypothetical protein
APZ15_20695	-1.0518235	0.00051336	LysR family transcriptional regulator
APZ15_32775	-0.9348605	0.00062602	universal stress protein UspA
APZ15_23290	-0.7714645	0.00067369	RND transporter
APZ15_11810	-1.0121409	0.00073253	hypothetical protein
APZ15_04910	-0.5927964	0.00093893	flagellar biosynthesis protein FlhF
APZ15_03025	-0.7108792	0.00109459	flagellar biosynthesis protein FlgB
APZ15_04740	-0.5932888	0.00113498	aminotransferase
APZ15_03030	-0.706266	0.0011765	flagellar biosynthesis protein FlgA
APZ15_03015	-0.5863948	0.00138716	flagellar biosynthesis protein FlgD
APZ15_02540	-0.6095042	0.00157553	8-amino-7-oxononanoate synthase
APZ15_11925	-0.8095754	0.00177245	capsid protein
APZ15_10615	-0.8564602	0.00180981	TonB-dependent receptor
APZ15_32750	-0.8358495	0.00183648	UDP-glucose 4-epimerase
APZ15_12465	-0.7032582	0.00210401	hypothetical protein
APZ15_02990	-0.5695306	0.0023691	flagellar biosynthesis protein FlgI
APZ15_32690	-0.8801278	0.00255246	universal stress protein UspA
APZ15_03235	-0.6191817	0.00266398	flagellar M-ring protein FliF
APZ15_32685	-0.8261255	0.0035032	universal stress protein UspA
APZ15_03020	-0.5933498	0.0035803	flagellar basal-body rod protein FlgC
APZ15_03000	-0.5659602	0.00391644	flagellar basal-body rod protein FlgG
APZ15_32755	-0.8088639	0.00392311	OsmY domain-containing protein
APZ15_10605	-0.848607	0.00396728	RNA polymerase subunit sigma
APZ15_32045	-0.6419302	0.00402385	hypothetical protein
APZ15_19255	-0.7101933	0.00469326	acyl-CoA dehydrogenase
APZ15_11915	-0.7530693	0.00478994	terminase
APZ15_23295	-0.7136653	0.00479821	transporter
APZ15_11980	-0.724408	0.00530614	hypothetical protein
APZ15_11960	-0.754902	0.00532469	phage tail protein
APZ15_23305	-0.9064728	0.00555347	alpha/beta hydrolase
APZ15_15610	-0.9155299	0.00562211	hypothetical protein
APZ15_15565	-0.9818148	0.00663219	hypothetical protein
APZ15_32720	-0.7204696	0.00701036	alcohol dehydrogenase
APZ15_09840	-0.5767857	0.00787882	multidrug DMT transporter permease
APZ15_03010	-0.5186136	0.0085112	flagellar biosynthesis protein FlgE
APZ15_15595	-0.8027645	0.00859944	hypothetical protein
APZ15_11930	-0.6992373	0.00909243	serine peptidase
APZ15_24145	-0.7169041	0.00998199	oligopeptide transporter ATP-binding component
APZ15_29025	-0.6215902	0.01022892	AraC family transcriptional regulator
APZ15_03005	-0.5094297	0.01028862	flagellar biosynthesis protein FlgF
APZ15_19250	-0.7039549	0.01160545	alkylhydroperoxidase
APZ15_03815	-0.5297868	0.0116385	transcriptional regulator
APZ15_15570	-0.7762557	0.01180211	baseplate assembly protein
APZ15_27885	-0.9150165	0.01182669	salicylate biosynthesis isochorismate synthase
APZ15_03230	-0.5569556	0.01235582	flagellar hook-basal body protein FliE

Gene	Log_2 fold change	Adjusted p-value	Annotated function
APZ15_32770	-0.8134205	0.01284411	hypothetical protein
APZ15_15575	-0.8807171	0.01299392	hypothetical protein
APZ15_03240	-0.53876	0.01306504	flagellar motor switch protein G
APZ15_30140	-0.5810739	0.01371687	RpiR family transcriptional regulator
APZ15_31900	-0.7538066	0.0137376	hypothetical protein
APZ15_15510	-0.8328544	0.01386561	hypothetical protein
APZ15_32605	-0.6804217	0.01448713	glutamyl-tRNA amidotransferase
APZ15_04140	-0.518451	0.01534227	flagellar motor switch protein FliN
APZ15_03250	-0.5010897	0.01574047	flagellar protein export ATPase FliI
APZ15_23300	-0.681919	0.0157919	hemolysin secretion protein D
APZ15_12015	-0.7503335	0.01602625	hypothetical protein
APZ15_30055	-0.5033759	0.01635426	hypothetical protein
APZ15_04735	-0.5475086	0.01740712	transferase
APZ15_32725	-0.7219855	0.01749977	nitroreductase
APZ15_13650	-0.6190911	0.01749977	2-nitropropane dioxygenase
APZ15_24790	-0.5560202	0.01749977	hypothetical protein
APZ15_11970	-0.7633595	0.01776252	hypothetical protein
APZ15_23620	-0.6589561	0.01776252	hypothetical protein
APZ15_15640	-0.6753861	0.01839132	virulence protein E
APZ15_11825	-0.7860047	0.01882027	hypothetical protein
APZ15_01730	-0.5667234	0.01882828	phasin
APZ15_32035	-0.6713348	0.01901296	metal ABC transporter ATPase
APZ15_27910	-0.8242034	0.01955513	non-ribosomal peptide synthetase
APZ15_22585	-0.5507485	0.01955513	hypothetical protein
APZ15_15550	-0.7666421	0.01977177	phage tail protein
APZ15_27280	-0.6920484	0.01987505	calmodulin-binding protein
APZ15_11765	-0.6279157	0.01995868	hypothetical protein
APZ15_02545	-0.5635843	0.02448592	dethiobiotin synthetase
APZ15_02995	-0.462292	0.02578117	flagellar biosynthesis protein FlgH
APZ15_17870	-0.4727179	0.0264617	sodium:proton antiporter
APZ15_29015	-0.5224725	0.02699779	APC family amino acid permease
APZ15_02550	-0.5399154	0.0272232	biotin synthase
APZ15_29020	-0.6208069	0.02774693	hypothetical protein
APZ15_04135	-0.6321834	0.02837945	flagellar biosynthesis protein FliO
APZ15_12000	-0.5905935	0.02892545	hypothetical protein
APZ15_32660	-0.6946013	0.03259813	alcohol dehydrogenase
APZ15_12030	-0.6029607	0.03259813	hypothetical protein
APZ15_11770	-0.6049316	0.03280121	hypothetical protein
APZ15_02945	-0.5010202	0.03338625	hypothetical protein
APZ15_36700	-0.4946904	0.03491788	peroxidase
APZ15_11815	-0.6873014	0.03621732	hypothetical protein
APZ15_00980	-0.4588859	0.03812413	peptidase
APZ15_01725	-0.6294077	0.04140059	acetyl-CoA acetyltransferase
APZ15_01720	-0.5778491	0.04594567	acetoacetyl-CoA reductase
APZ15_27900	-0.7397357	0.04597002	2,3-dihydroxybenzoate-AMP ligase
APZ15_22795	-0.7570074	0.046404	thioredoxin
APZ15_19675	-0.5147006	0.04968439	polyamine ABC transporter permease

Table S5. Iron-repressed genes in *B. cepacia* identified using RNA-seq. Biological triplicate cultures of *B. cepacia* WT were grown in LB-Miller broth with and without excess iron (1 mM $FeCl_3$) and total RNA was extracted for RNA-sequencing. Listed here are the genes which were significantly depleted (adjusted p-value < 0.05) in reads in the excess iron vs. baseline iron control. Genes were annotated according to the txid983594 NCBI genome file.

Gene	Log_2 fold change	Adjusted p-value	Annotated function
APZ15_06900	1.91420809	1.09E-39	FAD-dependent oxidoreductase
APZ15_07725	1.98170033	1.01E-32	FAD-dependent oxidoreductase
APZ15_28850	1.39793636	8.66E-18	aspartate carbamoyltransferase
APZ15_28575	1.50416225	3.00E-15	diaminopimelate decarboxylase
APZ15_15200	1.25721739	4.71E-15	RNA helicase
APZ15_10115	1.14366	3.99E-14	MFS transporter
APZ15_09900	1.08211004	6.89E-11	dihydrolipoamide dehydrogenase
APZ15_20500	1.37336253	3.31E-09	hypothetical protein
APZ15_31115	1.0337914	3.92E-09	cold-shock protein
APZ15_28135	0.9904126	1.16E-08	acyl-CoA dehydrogenase
APZ15_31120	1.08537958	2.91E-08	translation initiation factor IF-1
APZ15_30695	1.00955689	7.22E-08	purine nucleoside phosphorylase
APZ15_09885	0.94986809	9.13E-08	2-oxoisovalerate dehydrogenase
APZ15_09890	1.09980574	9.52E-08	2-oxoisovalerate dehydrogenase
APZ15_01455	1.01566335	1.20E-07	acetyl-CoA acetyltransferase
APZ15_09895	1.06521424	8.27E-07	branched-chain alpha-keto acid dehydrogenase subunit E2
APZ15_25535	0.96153372	1.00E-06	translation initiation factor IF-1
APZ15_28140	1.00359866	1.83E-06	AMP-dependent synthetase
APZ15_12565	0.84087118	2.27E-06	sulfate ABC transporter ATP-binding protein
APZ15_12555	1.00949874	2.40E-06	sulfate transporter
APZ15_08435	0.800105	2.48E-06	transglycosylase SLT domain protein
APZ15_07735	1.09002473	2.76E-06	4-hydroxybenzoate transporter
APZ15_00200	0.83722808	4.52E-06	phosphoadenosine phosphosulfate reductase
APZ15_30440	0.89420388	1.22E-05	peptidoglycan-binding protein
APZ15_07740	0.88938909	1.50E-05	homogentisate 1
APZ15_04995	0.7451365	1.82E-05	hypothetical protein
APZ15_25540	0.84004572	2.37E-05	hypothetical protein
APZ15_20505	0.90085441	2.37E-05	cold-shock protein
APZ15_29295	0.83463134	2.70E-05	hypothetical protein
APZ15_00210	0.71786018	3.75E-05	sulfite reductase
APZ15_00195	0.73293372	4.18E-05	sulfate adenylyltransferase
APZ15_12550	0.77478241	5.02E-05	sulfate transporter subunit
APZ15_17130	0.77365421	6.84E-05	hypothetical protein
APZ15_01585	0.7482411	0.00011614	disulfide bond formation protein DsbA
APZ15_00190	0.69756076	0.0001406	sulfate adenylyltransferase
APZ15_12570	0.77804634	0.00021567	transcriptional regulator
APZ15_00205	0.76562431	0.00025037	oxidoreductase
APZ15_14120	0.659274	0.00025064	50S ribosomal protein L31 type B
APZ15_05115	0.8608476	0.00033708	hypothetical protein
APZ15_22685	0.82683329	0.00043592	Rieske (2Fe-2S) protein
APZ15_33075	0.69301732	0.00049894	AMP-binding protein
APZ15_28145	0.77635369	0.0005245	methylmalonate-semialdehyde dehydrogenase
APZ15_24225	0.7395028	0.00057088	hypothetical protein
APZ15_12560	0.82100643	0.00065658	sulfate ABC transporter permease
APZ15_04765	0.69323257	0.00071667	30S ribosomal protein S21
APZ15_25530	0.68233789	0.00078172	cold-shock protein
APZ15_32350	0.68226213	0.00092037	acyl-CoA synthetase
APZ15_28160	0.83860479	0.00106075	enoyl-CoA hydratase
APZ15_29300	0.669446	0.00132381	inosine-5-monophosphate dehydrogenase
APZ15_31125	0.73478027	0.00133893	alpha/beta hydrolase
APZ15_01460	0.73326785	0.00154887	3-hydroxyacyl-CoA dehydrogenase
APZ15_07745	0.75770248	0.00156618	fumarylacetoacetase
APZ15_25525	0.80251477	0.00160611	hypothetical protein
APZ15_30655	0.66616312	0.00311753	nitrite reductase
APZ15_28150	0.84628258	0.00360042	3-hydroxyisobutyrate dehydrogenase
APZ15_01465	0.67011652	0.00390782	acyl-CoA dehydrogenase
APZ15_30445	0.57527007	0.00392311	ionic transporter y4hA
APZ15_01580	0.60280173	0.00396728	MarR family transcriptional regulator
APZ15_09010	0.5790859	0.00436471	electron transporter RnfB
APZ15_00430	0.64752188	0.00455114	50S ribosomal protein L33
APZ15_13000	0.73562275	0.00480821	MFS transporter
APZ15_15915	0.52577335	0.00492687	dihydrolipoamide dehydrogenase
APZ15_27260	0.59734348	0.00499272	aldehyde dehydrogenase
APZ15_01885	0.53313563	0.00524419	50S ribosomal protein L25
APZ15_28440	0.53304733	0.00636981	FAD-dependent oxidoreductase
APZ15_28570	0.94439741	0.006799	hypothetical protein
APZ15_26585	0.5999219	0.00689706	hypothetical protein
APZ15_00185	0.56291722	0.00800886	uroporphyrin-III methyltransferase

Gene	Log_2 fold change	Adjusted p-value	Annotated function
APZ15_36230	0.82298664	0.00806465	hypothetical protein
APZ15_33290	0.53504889	0.00890206	adenylosuccinate synthetase
APZ15_09015	0.50858239	0.01007729	electron transfer flavoprotein subunit beta
APZ15_01310	0.51344958	0.01007729	3,4-dihydroxy-2-butanone 4-phosphate synthase
APZ15_11075	0.53870635	0.01044698	hypothetical protein
APZ15_27520	0.5549963	0.01110281	amino acid permease
APZ15_11315	0.49799126	0.01120352	segregation and condensation protein B
APZ15_09525	0.50714508	0.01125699	glycosyl transferase
APZ15_27145	0.57652094	0.0113318	hypothetical protein
APZ15_09445	0.74671603	0.01201624	tRNA-Cys
APZ15_33335	0.51936803	0.01299392	flavodoxin
APZ15_01635	0.67032477	0.01386561	L-lactate permease
APZ15_20275	0.50559183	0.01536932	multidrug ABC transporter ATP-binding protein
APZ15_24875	0.49099683	0.01573621	L-threonine 3-dehydrogenase
APZ15_07810	0.57020705	0.0157919	aldehyde oxidase
APZ15_15260	0.55143005	0.01799658	tRNA-Glu
APZ15_15275	0.51358101	0.01839132	tRNA-Ala
APZ15_11265	0.67216077	0.01955513	tRNA-Pro
APZ15_00215	0.54171092	0.02019257	transcriptional regulator
APZ15_11215	0.61201103	0.02144406	50S ribosomal protein L35
APZ15_00130	0.62292681	0.02234378	hypothetical protein
APZ15_09005	0.5460626	0.02247356	metal ABC transporter substrate-binding protein
APZ15_30625	0.68911852	0.02312619	small multidrug resistance protein
APZ15_08685	0.5612836	0.02448592	tRNA-Arg
APZ15_38340	0.54335499	0.02479318	hypothetical protein
APZ15_23255	0.51858806	0.02497578	alpha/beta hydrolase
APZ15_29290	0.49620924	0.02522311	transcription elongation factor GreAB
APZ15_15910	0.52516335	0.02607509	Phasin (PHA-granule associated protein)
APZ15_14315	0.57472864	0.0273288	primosomal replication protein N
APZ15_33065	0.60943008	0.02774693	acetyl-CoA acetyltransferase
APZ15_00255	0.78034177	0.02818741	hypothetical protein
APZ15_01470	0.49761175	0.03050504	TetR family transcriptional regulator
APZ15_09345	0.54609289	0.03195523	anti-sigma factor
APZ15_03790	0.75848086	0.0343863	hypothetical protein
APZ15_14080	0.50346178	0.03447661	recombination protein RecR
APZ15_05140	0.45325301	0.03621732	orotate phosphoribosyltransferase
APZ15_00220	0.46722262	0.03812413	branched-chain amino acid ABC transporter
APZ15_09000	0.47130162	0.03812413	DL-methionine transporter permease subunit
APZ15_25510	0.64047535	0.03812413	hypothetical protein
APZ15_36235	0.72832502	0.03930393	hypothetical protein
APZ15_14305	0.51727277	0.04040064	50S ribosomal protein L9
APZ15_33330	0.43597969	0.04247427	TetR family transcriptional regulator
APZ15_15990	0.45178395	0.04252509	glutamine synthetase
APZ15_30615	0.46238751	0.04255752	4-hydroxybenzoate polyprenyltransferase
APZ15_28450	0.48053396	0.04437748	glutamine synthetase
APZ15_03960	0.72011341	0.0448486	hypothetical protein

Table S6. Iron-induced genes in *B. cepacia* identified using RNA-seq. Biological triplicate cultures of *B. cepacia* WT were grown in LB-Miller broth with and without excess iron (1 mM $FeCl_3$) and total RNA was extracted for RNA-sequencing. Listed here are the genes which were significantly enriched (adjusted p-value < 0.05) in reads in the excess iron vs. baseline iron control. Genes were annotated according to the txid983594 NCBI genome file.

Gene	Alternate ID	Log_2 fold change	Adjusted p-value
APZ15_04770	GGFLHMPP_00194	-0.1111778	0.90451401
APZ15_08735	GGFLHMPP_00997	0.13912742	0.91748674
APZ15_10485	GGFLHMPP_01354	-0.204374	0.6603581
APZ15_10615	GGFLHMPP_01381	-0.8564602	0.00180981
APZ15_12790	GGFLHMPP_01830	-3.325007	1.32E-99
APZ15_13410	GGFLHMPP_01957	0.14447575	0.90697819
APZ15_27935	GGFLHMPP_03614	-0.7151476	0.05463782
APZ15_27265	GGFLHMPP_03754	-1.2298424	6.27E-12
APZ15_27220	GGFLHMPP_03764	0.22152038	0.73069008
APZ15_27215	GGFLHMPP_03765	0.19488369	0.79398094
APZ15_23630	GGFLHMPP_04498	-1.0662754	0.00021923
APZ15_22715	GGFLHMPP_04684	-1.1368288	9.35E-09
APZ15_22220	GGFLHMPP_04786	0.22195034	na
APZ15_21665	GGFLHMPP_04897	-0.2766623	0.5573478
APZ15_20880	GGFLHMPP_05058	0.04293992	0.9741139
APZ15_19710	GGFLHMPP_05298	0.20919784	0.85813846
APZ15_18790	GGFLHMPP_05484	-2.1337118	3.95E-42
APZ15_31445	GGFLHMPP_05932	-0.0100092	0.99603049
APZ15_31265	GGFLHMPP_05969	0.09141988	0.93973097
APZ15_30000	GGFLHMPP_06227	-0.0493401	0.97595085
APZ15_28835	GGFLHMPP_06471	-2.2263542	4.46E-37
APZ15_34675	GGFLHMPP_06887	-0.9235059	1.35E-05
APZ15_35050	GGFLHMPP_06963	0.00671347	0.99603049
APZ15_35070	GGFLHMPP_06967	-0.0902081	0.9359056
APZ15_35470	GGFLHMPP_07045	0.02189847	0.99071542
APZ15_36965	GGFLHMPP_07356	0.02566865	0.99071542
APZ15_36995	GGFLHMPP_07363	0.08616953	0.95730836
APZ15_37180	GGFLHMPP_07399	-0.0724437	0.95741494
APZ15_37495	GGFLHMPP_07465	0.02978422	na

Table S7. Summary of RNA-seq data showing how excess iron affects the expression of genes encoding putative *B. cepacia* TBDTs. The log_2 fold change in read counts (excess iron/baseline iron) is shown for the 29 predicted *B. cepacia* TBDTs, with TBDT genes exhibiting significant changes (adjusted p-value < 0.05) highlighted in red. na indicates that there were insufficient data to calculate the adjusted p-value.

Regulated operon	Fur DNA-binding site sequence (5' to 3')
fagA-fumC-orfX-sodA	GAAAACAATAATCAATCTC
fagA-fumC-orfX-sodA	AATAATCAATCTCATTATC
pchR	GAGATTTATTATCATTGGC
pchR	GGAAATGAGATTTATTATC
pvdS	GTAATTGACAATCATTATC
phuSTUVW	CAAAACGCATATCTGAATC
phuR	GATAATTATTTGCATTAGC

Table S8. Experimentally-determined DNA-binding sites of the *P. aeruginosa* ferric uptake regulator (Fur) protein[11]. Some operons contain two Fur boxes upstream.

Strain	Genotype*	Source
Escherichia coli		
XL1-Blue	Wild-type	Lab strain stock
DH5α	Wild-type	Lab strain stock
CC118(λpir)	Wild-type	Zemer Gitai
S17-1(λpir)	Wild-type	Zemer Gitai
BL21	Wild-type	Lab strain stock
BL21(DE3) Δ*slyD*	Δ*slyD* mutant in BL21(DE3) background	Lab strain stock
Burkholderia cepacia		
ATCC 25416	Wild-type	ATCC
TD042	ATCC 25416 ΔGGFLHMPP_06959 (*tonB3*)::TpTer	This study
TD075	ATCC 25416 ΔGGFLHMPP_00610 (*fur*)::Km; pSCrhaB2-*fur*	This study
TD078	ATCC 25416 ΔGGFLHMPP_07362 (*tonB4*)::Km	This study
TD080	ATCC 25416; pSCrhaB2	This study
TD090	ATCC 25416 ΔGGFLHMPP_04892 (*tonB2*)::TpTer	This study
TD091	ATCC 25416 ΔGGFLHMPP_02544 (*tonB1*)::Km; pSCrhaB2-*tonB1*	This study
TD103	ATCC 25416 ΔGGFLHMPP_01381 (*pupB*)::Km	This study
TD118	ATCC 25416 ΔGGFLHMPP_01381 (*pupB*)::Km; pSCrhaB2-APZ15_10615 (*pupB*)	This study
TD122	ATCC 25416 ΔGGFLHMPP_00373 (*yddA*)::TpTer	This study
Other *Burkholderia cepacia* complex strains		
AU0158	*Burkholderia dolosa* AU0158 wild-type	John LiPuma
AU0756	*Burkholderia cenocepacia* AU0756 wild-type	John LiPuma
AU3578	*Burkholderia vietnamiensis* AU3578 wild-type	John LiPuma
AU15814	*Burkholderia multivorans* AU15814 wild-type	John LiPuma
AU21726	*Burkholderia vietnamiensis* AU21726 wild-type	John LiPuma
AU24362	*Burkholderia cenocepacia* AU24362 wild-type	John LiPuma
AU30438	*Burkholderia multivorans* AU30438 wild-type	John LiPuma
AMMD	*Burkholderia ambifaria* AMMD wild-type	John LiPuma
J2315	*Burkholderia cenocepacia* J2315 wild-type	John LiPuma
PC543	*Burkholderia dolosa* PC543 wild-type	John LiPuma

Table S9. Bacterial strains used in this study. *Abbreviations: TpTer, trimethoprim resistance; Km, kanamycin resistance. GGFLHMPP gene designations are derived from the ATCC 25416 genome file downloaded from the ATCC Genome Portal in the GenBank format. APZ15 gene designations are derived from the ATCC 25416 genome file (txid983594) downloaded from NCBI in the GenBank format.

Plasmid	Description*	Source
pRK2013	Triparental mating helper plasmid (Km^R, ColE1 ori)	Figurski and Helinski, 1979
pWC99	pQE-80L-P_{T5}:*uboA*-P_{mcjBCD}-*uboBCD* (Amp^R, ColE1 ori)	Cheung-Lee et al., 2020
pSCrhaB2	Rhamnose-inducible expression vector for *B. cepacia* (Tp^R, pBBR1 ori)	Cardona and Valvano, 2005
pYTK001	Template plasmid to amplify the PGphD promoter sequence (Cm^R, ColE1 ori)	Lee et al., 2015
pET28a-mCherry-CNA35	Template plasmid to amplify mCherry dropout reporter (Km^R, ColE1 ori)	Aper et al., 2014
p34E-Km	Template plasmid to amplify Km^R marker for allelic replacement in *B. cepacia* (Amp^R, ColE1 ori)	Shastri et al., 2017
p34E-TpTer	Template plasmid to amplify TpTer marker with transcription terminator for allelic replacement in *B. cepacia* (Amp^R, ColE1 ori)	Shastri et al., 2017
pSHAFT3	Suicide vector for one-step allelic replacement in *B. cepacia* (Amp^R, Cm^R, oriR6K)	Shastri et al., 2017
pRSFDuet-1	Plasmid for dual protein overexpression in *E. coli* (Km^R, RSF1030-derived RSF ori)	Lab strain stock
pTD005	pSHAFT3-ΔGGFLHMPP_06959 (*tonB3*)::TpTer to make *tonB3* knockout in ATCC 25416 (Amp^R, Cm^R, Tp^R, oriR6K)	This study
pTD013	pSCrhaB2-GGFLHMPP_00610 (*fur*) for *fur* overexpression (Tp^R, pBBR1 ori)	This study
pTD014	pSHAFT3 derivative compatible with Golden Gate cloning. pSHAFT3-Amp^R Cyt717Ade-BsaI-PGphD-mCherry-BsaI	This study
pTD016	pTD014-ΔGGFLHMPP_00610 (*fur*)::Km to knock out chromosomal *fur* in ATCC 25416 (Amp^R, Cm^R, Km^R, oriR6K)	This study
pTD017	pSHAFT3-ΔGGFLHMPP_07362 (*tonB4*)::Km to make *tonB4* knockout in ATCC 25416 (Amp^R, Cm^R, Km^R, oriR6K)	This study
pTD019	pTD014-ΔGGFLHMPP_02544 (*tonB1*)::Km to knock out chromosomal *tonB1* in ATCC 25416 (Amp^R, Cm^R, Km^R, oriR6K)	This study
pTD020	pSCrhaB2-GGFLHMPP_02544 (*tonB1*) for *tonB1* overexpression (Tp^R, pBBR1 ori)	This study
pTD022	pTD014-ΔGGFLHMPP_04892 (*tonB2*)::TpTer to make *tonB2* knockout in ATCC 25416 (Amp^R, Cm^R, Tp^R, oriR6K)	This study
pTD023	pTD014-ΔGGFLHMPP_01381 (*pupB*)::Km to make *pupB* knockout in ATCC 25416 (Amp^R, Cm^R, Km^R, oriR6K)	This study
pTD027	pRSFDuet-1-GGFLHMPP_02544 (*tonB1*) (Km^R, RSF1030 ori)	This study
pTD029	pSCrhaB2-APZ15_10615 (*pupB*) for *pupB* overexpression (Tp^R, pBBR1 ori)	This study
pTD030	pRSFDuet-1-GGFLHMPP_02544 (*tonB1*)-APZ15_10615 (*pupB*) (Km^R, RSF1030 ori)	This study
pTD031	pRSFDuet-1-APZ15_10615 (*pupB*) (Km^R, RSF1030 ori)	This study
pTD032	pTD014-ΔGGFLHMPP_00373 (*yddA*)::TpTer to make *yddA* knockout in ATCC 25416 (Amp^R, Cm^R, Tp^R, oriR6K)	This study

Table S10. Plasmids used in this study. *Abbreviations: TpTer/Tp/Tp^R, trimethoprim resistance; Km/Km^R, kanamycin resistance; Amp^R, ampicillin resistance; Cm^R, chloramphenicol resistance. GGFLHMPP gene designations are derived from the ATCC 25416 genome file downloaded from the ATCC Genome Portal in the GenBank format. APZ15 gene designations are derived from the ATCC 25416 genome file (txid983594) downloaded from NCBI in the GenBank format.

Primer	Sequence (5’ to 3’)
oTD011	GCTCGAATTCTATGCACGAACCCAGTTGACATAAGCCTGTTCGGTTC
oTD012	CCTACTCTCTTAGGCCACACGTTCAAGTG
oTD013	GTGTGGCCTAAGAGAGTAGGGAACTGCCAG
oTD014	GCTCGAATTCGACAGGAAGAGTTTGTAGAAACGCAAAAAGG
oTD015	CGACTCACTATAGGGAGACC
oTD016	TACAAGCTTGCATGCCTG
oTD025	TTTAATCTAGAAACCTTGTCGATCGACGC
oTD026	TTTAAGAATTCACGACACGTTCGTCGAATC
oTD027	TTTAAGGTCTCGCATAGTTCGGTCCAGTTGCAAC
oTD028	TTTAAGGTCTCCTGTCTTCAACCTGAACTGACGGG
oTD029	TAATTGGTCTCTTATGCACGAACCCAGTTGAC
oTD030	TTTAAGGTCTCTGACAGGAAGAGTTTGTAGAAACGC
oTD035	GTAACGAGAAGGTCGCGAATTC
oTD036	CGCTTCTGCGTTCTGATTTAATCTG
oTD039	CTTCAGCTGATGTGTGATAACATACTG
oTD040	ATGTAACGCACTGAGAAGCC
oTD043	AATACCAACCGACGACTTGAC
oTD044	ACAAGAAGGATTCGACATGGG
oTD045	TGTTCGACACCGATCCG
oTD046	TCCATCACGGACTTGTTGC
oTD062	TTTCAACATCTTCCCGGACG
oTD063	ACGATGGAACTGGAACTGC
oTD064	TTTAAGGTCTCTACCGCGAGAACCACG
oTD065	CTGACAACGATCGGAGGAC
oTD068	TTTAATCTAGAGTGATAGATGCCCCACACC
oTD071	TTTAAGAATTCATTAAGGCGGTGTGCGAG
oTD076	TTTAAGAATTCGAGACCGAAAGTGAAACGTGA
oTD078	CCTTAGAAACCATCTAGTATTTCTCCTCTTTCTCTAGTAGCTAAG
oTD079	GAGGAGAAATACTAGATGGTTTCTAAGGGCGAAGAG
oTD080	TTTAATCTAGAGAGACCTCACTTGTATAACTCATCCATGCCACC
oTD083	TTTAACATATGACCAATCCGACGGATCT
oTD084	TTTAAGGATCCTCAGTGCTTGCGGTGC
oTD089	TCATGATCAGCGCCTCAC
oTD090	GGCGAGAGACTTTATCGATCG
oTD091	CCTCGTCTTGGAGTTCATTCAG
oTD092	GGTCTTGTCGATCAGGATGATC
oTD093	ACTGGGCACAACAGACAATC
oTD094	TTTAAGGTCTCGAATTTTCGCATCCTGGTCGAC
oTD095	TTTAAGGTCTCTCCATCGGATTGGTCATGGCTAGG
oTD096	TTTAAGGTCTCGACGCCAAGCACTGAAATCCAGCC
oTD097	TTTAAGGTCTCTCTAGGCCATTTTCCGATCAACGG
oTD098	TTTAAGGTCTCGATGGACAGCAAGCGAACC
oTD099	TTTAAGGTCTCTGCGTCGAATTAATTCCGCGA
oTD104	TTGCTGTCCATCTTCAGTCCCCCAACGC
oTD105	ATTCGACGCAACCTGAACTGACCCGTG
oTD106	GGACTGAAGATGGACAGCAAGCGAACC
oTD107	CAGTTCAGGTTGCGTCGAATTAATTCCGCGA
oTD114	TTTAAGGTCTCGAATTCTTTCAAGGGTCCGATGACC
oTD115	TTTAAGGTCTCTCCATGATTCATACGTGGTGCAGC

oTD116	TTTAAGGTCTCGACGCCCCGTCATATTCGGTAGATTCAAAG
oTD117	TTTAAGGTCTCTCTAGAGAATGCGGTCATGATCAGC
oTD118	ACCATTTCCTCGCCGAAC
oTD119	CCGGTGTTCATTGCCATG
oTD120	TTTAACATATGAATCCCCGAGTGATCG
oTD121	TTTAAGGATCCTTAGTCGTCGAGCGTGAAG
oTD128	TTTAAGGTCTCGAATTCGAAAGGTTTCCTGAGCGC
oTD129	TTTAAGGTCTCGCATAGCTCGACTCCTTGCAATGA
oTD130	TTTAAGGTCTCCTGTCGATTTCAAGCTGGGCTGAC
oTD131	TTTAAGGTCTCTCTAGACGCACGAAGAAGTTGTACG
oTD132	AACGAGTTCCAGGCGATC
oTD133	GAAATCGGTTGCGAACGC
oTD134	TTTAAGGTCTCGAATTCTCGTCCTCGATACCGACA
oTD135	TTTAAGGTCTCTCCATCATGATCGGGAAGTCGGT
oTD136	TTTAAGGTCTCGACGCCAGTTCTGACCACGCAATC
oTD137	TTTAAGGTCTCTCTAGATTCGTAGTCCATCGTCGC
oTD142	GTGTTGCGTTCCGACATC
oTD143	GACGAGCCGTTCAACAAC
oTD144	TATTCGCAGTCGCTGAAGC
oTD151	AAGCGTTATCTCGTGAAGGC
oTD152	ACCTTGGACACCATACGTTC
oTD168	TTTAAGGTCTCCCATGAATCCCCGAGTGATCG
oTD169	TTTAAAAGCTTTTAGTCGTCGAGCGTGAAG
oTD170	TTTAACCTAGGTCAGAACTGCAGCGTGG
oTD172	ATGATCGTGTCCTGCGTG
oTD173	TTTAACATATGGGTAATGAAAGCAGCACTG
oTD174	TTTAAAAGCTTTCAGAACTGCAGCGTGG
oTD175	TTTAAGGTCTCGAATTCTGTCCCGGACGTTCATC
oTD176	TTTAAGGTCTCGCATAGTGCTTCCTGATGAGTAGCC
oTD177	TTTAAGGTCTCCTGTCGTGAACGACAGCGACAAG
oTD178	TTTAAGGTCTCTCTAGAGACTGCGTTGACGGTGAT
oTD179	TCGATGTGGACGATCTGC
oTD180	CTGGAAGAAAACGGGTTCG
pRSFDuet-MCS1-F	CCCCTGTAGAAATAATTTTGTTTAACTTTAATAAGG
pRSFDuet-MCS1-R	CGATTATGCGGCCGT
pRSFDuet-MCS2-F	ATAACAATTCCCCATCTTAGTATATTAGTTAAGTATAAG
pRSFDuet-MCS2-R	GTTATGCTAGTTATTGCTCAGCG

Table S11. Oligos used in this study.

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