Supplementary Information

Bismuth drug eradicates multi-drug resistant *Burkholderia cepacia complex* via aerobic respiration

Jingru LI[a,b], Haibo WANG[a], Peng GAO[c], Runming WANG[a], Chun-Lung CHAN[a], Richard Yi-Tsun KAO[d], Hongyan LI[a,*] and Hongzhe SUN[a,*]

[a]*Department of Chemistry, The University of Hong Kong, Hong Kong SAR, P.R. China.*

[b]*Chemistry and Chemical Engineering Guangdong Laboratory, Shantou 515031, P.R. China.*

[c]*Applied Oral Sciences & Community Dental Care Division, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, P.R. China.*

[d]*Department of Mircobiology, The University of Hong Kong, Hong Kong SAR, P. R. China.*

*Corresponding author: H. Sun (hsun@hku.hk)

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Supplementary methods

RT-PCR and RNA extraction

RNA extraction

B. cepaica 6349 was grown to a mid-log phase (OD_{600}= 0.5) and treated with different concentrations of CBS (0, 12.5, 25, 50 μg/mL) for 4 h, followed by washing by 1x PBS for three times to remove extra CBS. The bacteria were resuspended by 10 mg/mL lysozyme and incubated at 37°C for 1h and collected by centrifugation at 12000 rpm for 15 min. 500 μL supernatant was collected from each sample and was mixed with 1 mL RNAiso Plus (Takara) by vertexing vigorously until complete mix and then stand for 5 min at room temperature (RT). Next, 200 μL chloroform (ACS grade) was added into the mixture, followed by standing for 5 min at RT. The mixture was subsequently centrifuged at 4°C, 1200 rmp for 15 min, and the mixture was demixed into three phases. The

liquid (300 µL) from the top water phase was transferred into a new 1.5 mL EP tube and mixed with equal volume of isopropanol (Sigma-Aldrich, ACS reagent) and stood for 10 min at RT, followed by 15 min centrifuge at 4°C. The supernatant was discarded, and the precipitate was washed by 75% EtOH for 2 times. After the last centrifugation, the precipitate was dry in air for 10 min and resuspended with 25 µL 0.1% diethyl pyrocarbonate (DEPC) H_2O and stored at -80°C.

Quantitative real-time PCR

The extracted total RNA was first reverse-transcribed into complementary DNA (cDNA) by PrimeScript (Takara RR036A). The primers used in quantitative real-time PCR were designed with NCBI Primer-BLAST (blast.ncbi.nlm.nih.gov) and synthesized by BGI Genomics Co., Ltd. The sequences of primers are listed in the Table S6. The real-time PCR reactions were carried by SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara RR820A) on StepOne ™ Real-time PCR system (Applied Biosystems) by following the instruction of the manufacturer. The house keeping gene *gyrB* (encoding the B subunit of DNA gyrase) was selected to be the reference gene as negative control and the expression levels of target genes were calculated by $\Delta\Delta C_T$ method. The relative units of mRNA of target genes were calculated by the equation as follow:

$$\textbf{Relative expression of target gene} = 2^{-\Delta\Delta C_T}.$$

The construction of overexpression strain and plasmid

Plasmid construction

The *cyoC* and *cydA* overexpression plasmids were constructed based on the vector pMLBAD which was designed by Lefebre et al.[1] and purchased from addgene (#32056). The DNA fragment of *crp* was using the genome of *B. cepacia 6349* as template and amplified by PCR. The plasmid was first digested by two restriction enzymes, Xbal (Thermo Scientific™) and HindIII (Thermo Scientific™) at 37°C for 1 h and purified by MiniBEST DNA Fragment Purification Kit Ver.4.0 (Takara, 9761).

Conjugation

The single colonies growing on the plate were verified by enzyme digestion to prevent contamination of competent cell. The correct S17 strain was inoculated in fresh LB liquid medium containing 50 µg/mL trimethoprim, while the *B. cepacia* 6349 was inoculated in fresh BHI liquid medium at 37°C and 250 rpm overnight. On the next day, the overnight culture of S17 and *B. cepacia* 6349 were transferred into fresh LB liquid

medium at a ratio of 1:100. Both of *E. coli* S17 and *B. cepacia* 6349 were incubated to OD_{600} around 1. Using as the donor bacteria, *B. cepacia* 6349 served as recipient bacteria to conjugate.

Bacterial suspensions of *E. coli* S17 and *B. cepacia* 6349 were taken into 1.5 mL centrifuge tube and centrifuged at 12,000 rpm for 1 min to collect bacteria. 1 mL fresh LB medium was added to resuspend the bacterial to remove the used medium and antibiotics. The washing step was repeated for 2 times. Taking OD_{600} = 1.0 as the bacterial concentration reaching 1×10^9 CFU/mL, the ratio of *E. coli* S17 to *B. cepacia* 6349 was calculated to 3:1 to 10:1 and the total bacteria number was less than 3×10^9 CFU.

A small filter device was connected to the vacuum filter pump. The appropriate amount of 75% alcohol was added into the filter device for disinfection, and the vacuum pump was turned on to remove all the liquid. The washing step by 75% alcohol was repeated for two times and the pre-sterilized 0.22 μm cellulose nitrate filter was placed in the filter and reassembled. The mixed bacterial solution was transferred to the center of the filter membrane, and the vacuum pump was turned on to remove the medium and leave the mixed bacterial on the filter membrane. Next, the filter membrane was carefully removed and placed on a nutrient agar plate (without antibiotics) with bacteria side up at 37 °C for 8-12 h.

On the next day, the filter membrane was carefully transferred into a 1.5 mL centrifuge tube with the addition of 1 mL fresh LB medium, followed by vortexed for 30 s to wash bacteria off from the filter membrane. The eluted bacterial solution was diluted by 10 and 100 folds, and the original concentration, 10x and 100x dilution were spread on the 100 μg/mL trimethoprim/ 50 μg/mL kanamycin BHI agar plate at 37 °C for 24-48 h until a single colony grows on the plate.

***Galleria mellonella* infection model**

G. mellonella larvae were divided into three group and each group contained 8 larvae. $1x10^5$ CFU *B. cenocepacia* J2315 into the larvae of *G. mellonella* at the right post leg. After 1 h post- infection, *G. mellonella* was treated with vehicle (PBS), low dose of CBS (10mg/kg) and high dose of CBS (20 mg/kg) by infected 5 μL at left post leg. The infected *G. mellonealla* were observed for 5 days to count the survival rate and larvae used for bacterial load counting were collected after 24 h treatment.

The synthesis of complex used in screening

The bismuth complexes used in this study, i.e., $Bi(Hino)_3$, $Bi(GSH)_3$, $Bi(Tro\text{-}NH_2)_3$, and $Bi(TS)_3$, were synthesized similarly as reported by Wei *et al.* [2] The synthesis of $Bi(NAC)_3$, Bi(DTT)OTf and $Bi(4\text{-}PySH)_3(NO_3)_3$ were described below. $Bi(GSH)_3$ and $Bi(NAC)_3$ are soluble in water, while the other compounds are soluble in DMSO.

$Bi(NAC)_3$

$Bi(NAC)_3$ was synthesized with a modification method from previous report.[2] 0.816 g (5 mmol) of N-acetyl cysteine (NAC) was dissolved in 40 ml ethanol and 5 ml 28% ammonia solution, bismuth subcarbonate 2.55 g (5 mmol) was added to the solution. The suspension was refluxed for 4 h and slowly turning yellow. The unreacted bismuth (III) subcarbonate was then filtered off. The yellow solution was blew with compress air to remove ammonia for 1 h, the resulting yellow solid was then filtered to yield $Bi(NAC)_3$ (1.00 g, 86.2% yield).

^{1}H NMR (400MHz, D_2O), δ (ppm): 1.9 (s, 9H), 4.1 (dd, J1= 4 Hz, J2= 12 Hz, 3H), 4.3 (dd, J1= 16 Hz, J2= 8 Hz, 3H), 4.4 (t, J= 8 Hz, 3H). ^{13}C NMR (400 MHz, D_2O), δ (ppm): 22.2, 31.0, 57.6, 173.6, 176.7. HR-ESI-MS: found m/z = 531.0082 $[Bi+2NAC-H]^-$, calc. for m/z 531.0091 $[C10H15N2O6S2Bi-H]^-$. TGA analysis: 68.69% weight loss at 550°C in compress air, proposed structure: $[Bi(NAC)_3](NH_4)_3$.

Bi(DTT)(OTf)

To a solution of dithiothreitol (0.386 g, 2.5 mmol) in 40 ml methanol, bismuth(III) triflate (1.64 g, 2.5 mmol) was then added to the solution and stirred for 2 h to allow all bismuth(III) triflate to dissolved yielding a yellow solution. The volume of the solution was then reduced to minimum which still can dissolve the complexes and recrystallized by addition of diethyl ether. The yellow solid was then filtered and give Bi(DTT)(OTf) as bright yellow solid (0.816 g, 64% yield).

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 3.3 (s, 4H), 3.9 (s, 2H), 4.1 (bs, 1H), 5.4 (bs, 1H). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 119.5, 122.7. ^{19}F NMR (400 MHz, DMSO-d_6), δ (ppm): 77.7.

$Bi(4\text{-}pySH)_3(NO_3)_3$

To a solution of 4-mercaptopyridine (0.5 g, 4.5mmol) in 30 ml ethanol, bismuth(III) nitrate pentahydrate (0.727 g, 1.5 mmol) was then added to the solution and stirred for 2 h to allow all bismuth(III)nitrate to dissolved yielding a yellow to red solution. The solution was then concentrated and recrystallized in water and acetone. The red solid was then filtered and gave $Bi(4\text{-}pySH)_3(NO_3)_3$ as predicted structure (0.73 g, 67% yield).

^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.3 (s, 6H), 7.8 (s, 6H),12.8 (bs, 3H). ^{13}C NMR (400 MHz, DMSO-d_6), δ (ppm): 130.8, 136.7. TGA analysis: 59.67% weight loss at 550°C in compress air, proposed structure: $Bi(4\text{-}pySH)_3(NO_3)_3$.

UV-vis spectroscopy

UV-vis spectroscopy was modified from [3]. In brief, 1 mM $(Bi(NTA)_3$ were stepwise titrated into BcMDH (10 µM) in a titration buffer (20 mM Tris-HCl, 0.15 M NaCl at pH 7.0) and UV-vis spectra were recorded in a range of 220–

600 nm at least 30 min after each addition. The binding of Bi^{III} to BcMDH was monitored by the increase in absorption at 340 nm due to LMCT. The UV titration curve was fitted to Ryan–Weber nonlinear equation[4]:

$$\boldsymbol{I = \frac{I_{max}}{2C_p}\left[(K_d + C_m + C_m) - \sqrt{(K_d + C_m + C_p)^2 - 4C_mC_p}\right].}$$

I, I_{max}, C_p and C_m represent UV absorbance intensity, maximal UV absorbance, the total concentrations of proteins and those of ligands, respectively; K_d is the dissociation constant. For Bi^{3+}, the dissociation constant from BcMDH was derived by $K_d{}' = K_d/K_a$, where K_d is the dissociation constant of $Bi(NTA)_3$ from NDM-1 determined from Ryan–Weber nonlinear fitting[3] and K_a is the formation constant of $Bi(NTA)_3$ with $\log K_a$ = 17.55.

In vitro BcMDH activity measurement

The activity of BcMDH was modified from.[5] In brief, BcMDH in its buffer were incubated with different molar equivalents of CBS for 2 h at room temperature. The proteins treated with CBS were diluted to a proper concentration for enzymatic activity by Malate Dehydrogenase Activity Assay Kit (ab183305). The absorbance at 450 nm was monitored constantly for a duration of 20 min. A standard curve was constructed using five concentrations of NADH ranging from 25 to 500 nmol for calculation.

Bliss model of synergy

The calculation of synergy was followed by the formula[6]:

$$\boldsymbol{S = (f_{x0}/f_{00})(f_{0y}/f_{00}) - (f_{xy}/f_{00}).}$$

f_{xy}, f_{x0} and f_{0y} represent the growth rate of bacteria in the presence of the combined drugs and individual drugs, x and y respectively. f_{00} represents the growth rate of bacteria in the control group.

Time-kill kinetics and persister model

The time-kill assay was modified from.[7] In brief, mid-log phase of *B. cepacia* bacterial cells were treated with different drugs (antibiotics and CBS) for different incubation times and serially diluted by 1x PBS. The bacterial content was confirmed by enumerating on agar plate. The persister model was modified from.[8] *B. cepacia* overnight culture was diluted in BHI and grown to OD_{600} of 0.2, followed by 1000-fold dilution in 50 mL BHI and grown for 16 h at 37 °C with 220 rpm shaking to obtain stationary phase cells. Cells were then treated with 5 μg/mL ciprofloxacin for 4 h, and the remaining persister cells were washed with PBS, resuspended in BHI and adjusted OD_{600} to around 0.2. Growth of the persister cells exposed to combination therapy in 96-well plate was monitored for 16 h.

Biofilm killing assay

B. cepacia bacterial cells were cultured in BHI medium at 37°C for 16 h and reached an OD_{600} around 1, followed by diluted by BHI to OD_{600}=0.0025. Bacterial suspension was added into 96-well MBEC biofilm inoculator (19113, Innovotech Inc.) for 8 h to allow bacteria to attach to the surface of the inoculator. The supernatant was replaced with fresh BHI medium for 16 h incubation at 37°C. The inoculator was gently washed by PBS for three times and then added fresh BHI with indicated concentration of combination drugs for 24 h incubation. Next, biofilms were washed by PBS for three time, followed by dispersed by sonication for 45 min. The bacterial content was enumerated by agar plating. All these tests were performed in triplicate.

Cell viability

A549 cells were purchased from ATCC and were grown in 96-well cell culture plate till 70% confluency in DMEM (high glucose) medium. Bi^{III}-containing drug combinations were incubated with A549 cells for 24 h and then added 80 µL XTT working solution (Cell proliferation Kit II, Roche) into each well and incubated for 2 h at 37°C. The cell viability was monitored by microplate reader (SpectraMax iD3, Molecular Devices) at OD562 nm. All these tests were performed in triplicate.

Oxygen consumption

The bacterial oxygen consumption assay was examined with the extracellular oxygen consumption assay kit (ab197243, Abcam). *B. cepacia* bacterial cells were cultured in BHI medium at 37°C for 16 h and reached an OD_{600} around 1, followed by adjusting the bacterial content to $1x10^6$ CFU/mL with indicated concentration of co-therapies. 150 µL bacterial suspension and 10 µL O_2 probe were added into each well of 96-well plate and covered by a thin layer of mineral oil for air isolation. The fluorescence signal was monitored by microplate reader (SpectraMax iD3, Molecular Devices) at an excitation/emission of 380/650 nm every 10 min for 24 h. All the tests were performed in triplicate.

Bi^{III}-binding protein identification

Protein extraction and Bi^{III} content measurement

Bi^{III}-treated bacterial cells were harvested at different time points by centrifugation at 4500 g and 4 °C for 10 min, followed by washed with cold 10 mM Tris (100 mM NaCl and pH 7.5) buffer for three times. Collected pellets were resuspended in the same buffer containing 1 mM TCEP and 0.5 mM PMSF, and then lysed through

sonication (amplitude: 20%, 5 sec on, 20 sec off, in total 5 min on the ice-water bath) and spun at 4500 g and 4 °C for 15 min to get the supernatant, which was further fractioned by spinning at 100000 g and 4 °C for 10 min. Cytosolic proteins were harvested from the supernatant, while the membrane proteins were dissolved from the pellets with Tris buffer containing 1% (w/v) SDS.

Protein concentrations and Bi^{III} contents in cytosolic and membrane fractions were detected by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) and ICP-MS, respectively. Bi^{III} content was normalized to protein concentration to get the ratio of Bi^{III}/protein. For the distribution of Bi^{III} in cytosolic and membrane fractions, same amounts of *B. cepacia* cells were prepared for measurement of total Bi^{III} content, the percentages were obtained by dividing the Bi^{III} content in different fractions to total Bi^{III} content.

Separation and identification of Bi^{III}-binding proteins with GE-ICP-MS

The concentration and length of freshly prepared gel column were optimized with the extracted proteins containing iodine-labelled standard proteins as molecular weight (MW) markers. To cover the broad MW range of Bi^{III}-binding proteins, two gel columns with different concentration were utilized for separation. In detail, a reverse gradient gel column, with concentration of 20% (1.7 cm) and 18% (2.0 cm) was used to separate proteins with MW less than 20 kDa, while another one with concentration of 15% (1.7 cm) and 12% (2.0 cm) to separate proteins with MW higher than 20 kDa. 4% stacking gel of 1.2 cm were utilized. A two-step voltage program with a stacking procedure of 60 min at 200 V, and the resolving at 600 V was applied for the separation. The elemental detection by ICP-MS was started in the beginning of the second step. For each GE-ICP-MS experiment, 0.5 µg I-RA, 1 µg I-CA, 2 µg I-OVA, 1 µg I-BSA and 2 µg I-OTF were added as internal standards for the calibration of MWs and intensity of Bi^{III}-binding proteins.

The proteins in the collected fractions after gel electrophoresis separation were identified through peptide mass fingerprinting. The destained gel pieces were first rinsed with MilliQ water, 50% acetonitrile (ACN), then by 10 mM ammonium bicarbonate and dehydrated with 100% ACN. The gel plugs were rehydrated with 12.5 ng/µL trypsin (Promega) with volume that was sufficient to cover the gel pieces (e.g. ~ 50 µL) in 10 mM ammonium bicarbonate. After incubation for 16 h at 37°C, the peptides were extracted twice using 5% formic acid (FA)/50% ACN and then extracted once with 100% ACN. After drying in a SpeedVac concentrator (Eppendorf) the peptides were resuspended in 0.1% FA and purified using µC-18 Ziptips (Millipore). The desalted peptides were then mixed in a 1:1 ratio with 10 mg/mL α-cyano-4-hydroxycinnamic acid matrix (Fluka) dissolved in 0.1% FA/50% ACN.

Protein identification and characterization was performed using the 4800 MALDI TOF/TOF Analyzer (ABSciex) which was equipped with a Nd:YAG laser that operates at 355 nm to ionize the samples. All mass spectra were acquired in positive ion Reflector mode using the 4000 Series Explorer version 3.5.28193 software (ABSciex).

Before each job run, the MS and the MS/MS were calibrated using the peptide calibration standard, 4700 Cal-Mix (ABSciex), which was spotted across 13 locations on the MALDI target plate. Characteristic spectra were attained by averaging 500 acquisitions in Reflector mode and in MSMS mode with the smallest amount possible laser energy in order to keep the best resolution. Precursor ions with a charge state of 1+ were fragmented through Post-source Decay (PSD). First, each sample was analyzed with MALDI-TOF MS to create the PMF data (Scanning range: 900-4000 m/z). Next the five most abundant peptides (precursors) that were not on exclusion list were chosen for advance fragmentation (MALDI-TOF/TOF) analysis to make the full scan mass spectrum. For PMF, the peak detection criteria used were a minimum S/N of 5, a local noise window width mass/charge (m/z) of 250 and a minimum full-width half maximum (bins) of 2.9. The following monoisotopic precursor selection criteria were used for MSMS: minimum S/N filter of 20, excluding the most commonly observed peptide peaks for trypsin and keratin, and excluding the precursors within 200 resolutions. The peak detection criteria for MSMS used were an S/N of 5 and a local noise window width of 250 (m/z) and a minimum full-width half maximum (bins) of 2.9.

The combined PMF and MS/MS search was then performed using GPS Explorer algorithm version 3.6 (ABSciex) against the non-redundant NCBInr database using the in-house MASCOT search engine version 2.2. MASCOT search analysis settings were as follow: Fixed modification: carbamidomethyl (C); variable modification: oxidation (M) MSMS fragment tolerance: 0.2 Da; precursor tolerance: 75 ppm, Peptide charge: +1; Monoisotopic. In a [PMF+MS/MS] analysis, Protein Score was a combination of PMF-type protein score and total ion score. The criteria for protein identification were based on the probability score of each search result. Significant matches had scores greater than the minimum threshold set by MASCOT (with $P<0.05$).

BcMDH overexpression

A single colony of *E. coli* BL21(DE3) transformed with BcMDH was inoculated into LB medium supplied with 50 µg/mL kanamycin and grown at 37 °C. Protein overexpression was induced using 0.2 mM IPTG at OD_{600} around 0.6. The bacterial culture was incubated at 25 °C overnight. To purify the respective protein, the cultured cells were harvested by centrifugation at 4,500 × *g* and resuspended in a lysis buffer (20 mM Tris-HCl, 0.5 M NaCl, and 1 mM PMSF at pH 7.0). The cells were ice-cooled and lysed by sonication and then centrifuged at 35,000 × *g* for 30 min to remove the majority of cell debris. The supernatant was filtered using Minisart syringe filter (0.45 µm) to remove any remaining large and insoluble cell debris, and was then applied to a 5 mL Ni(II)-loaded HiTrap chelating columns (GE Healthcare) at a rate of 2 mL/min. The column was washed using five column volumes of washing buffer (20 mM Tris-HCl, 0.5 M NaCl, and 10 mM imidazole at pH 7.0). The protein was eluted out using four column volumes of the same buffer with gradient amounts of imidazole and was

subsequently dialyzed against the cleavage buffer (20 mM Tris-HCl, 0.15 M NaCl at pH 7.0). The N-terminal His-tag of the fusion protein was cleaved by adding 50 NIH units of thrombin at 4°C for overnight with mild shaking and the cleaved His-tag was separated from the resulting proteins by passing through the Ni(II)-NTA column again using washing buffer so that >90% of the proteins were in the flow-through fraction. The purity of the protein was 98%, identified by MALDI-TOF MS.

Non-competitive inhibition

BcMDH (200 nM) was incubated with CBS (0, 5, 10, 20, 40 μg/mL) for 1 h at 25 °C with gentle shaking. The assay was performed in a 96-well microplate reader at RT. The final assay buffer contains 50 mM Tris-HCl at pH 7.0, 150 mM NaCl, with NADH and oxaloacetate (OAA) (50mM, 10 μL) as the substrate ranging from 10 to 150 μM. Control experiment was also performed in the absence of inhibitors under the same conditions. The K_m and V_{max} for both the uninhibited and inhibited reactions were obtained by fitting the data into the double reciprocal Lineweaver–Burk plots.

Succinate level

The bacterial lysis was treated in the same method with enzymatic assays. The succinate level of bacterial cell was examined with succinate assay kit (abcam204718). The bacterial sample was treated as the instruction of manufacture's, incubated at 37°C for 30 min and read OD at 450 nm.

Membrane integrity

B. cepacia bacterial cells were cultured in BHI medium at 37°C for 16 h and reached an OD_{600} around 1, followed by diluted by BHI to OD_{600} around 0.5. Bacterial cells were gently washed by PBS for three times and then resuspended in PBS. PI was added into bacterial suspension and the final concentration was adjusted to 1 μM. Next, CBS with indicated concentration was added into the mixture and then monitored the fluorescence at an excitation/emission of 535/615 nm every 10 min for 2 h at 37°C with microplate reader (SpectraMax iD3, Molecular Devices). All these tests were performed in triplicate.

GFP expression analysis

The GFP expression evaluation was modified from [1]. In brief, the mid-log phase of GFP-expressed *B. cepacia* strain were treated with different concentrations of CBS or treated with 50 μg/mL CBS for different times. The collected bacterial cells were wash by 1x PBS for three times and transferred into 96-well plate. The plate was read fluorescence at $\lambda_{ex}/\lambda_{em}$= 485/510.

Supplementary figures and tables

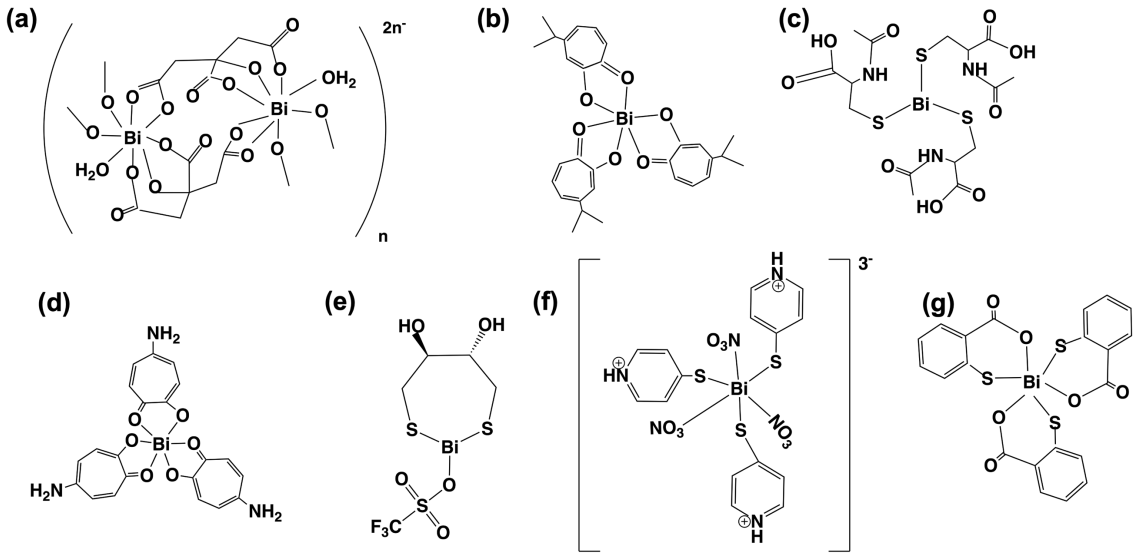

Figure S1. Proposed chemical structures of bismuth compounds used in this study. (a) CBS [3], (b) $Bi(Hino)_3$ [2], (c) $Bi(NAC)_3$ [9], (d) $Bi(Tro\text{-}NH_2)_3$ [2], (e) Bi(DTT)OTf, (f) $Bi(4\text{-}PySH)_3(NO_3)_3$ and (g) $Bi(TS)_3$ [2].

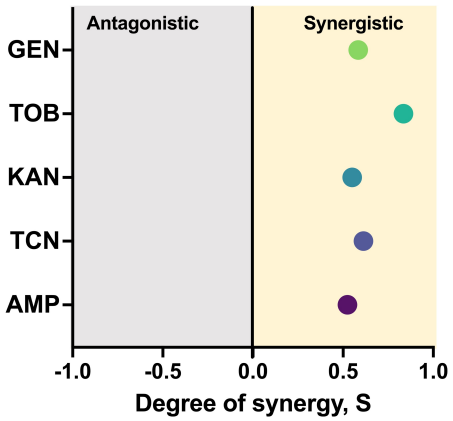

Figure S2. The Bliss model for synergy demonstrating the synergistic effects between CBS (12.5 μg/mL) and different antibiotics.

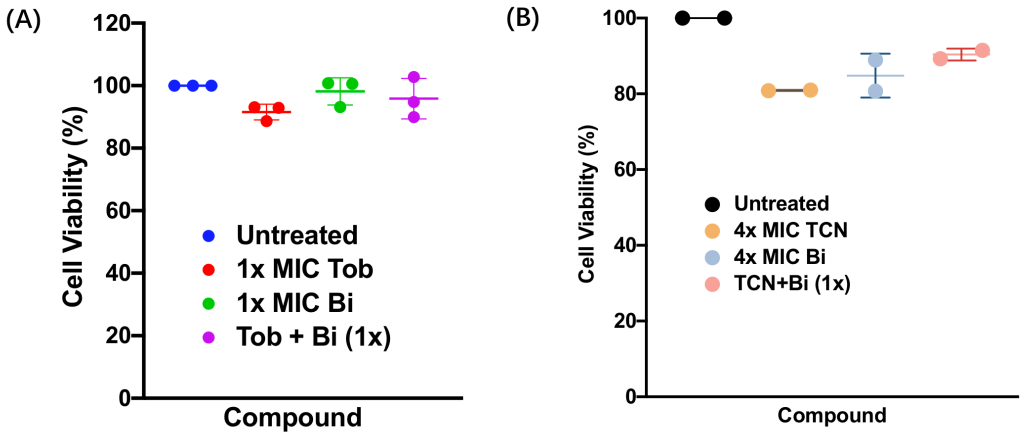

Figure S3. Cytotoxicity of the combination of tobramycin (A)/ tetracycline (B).

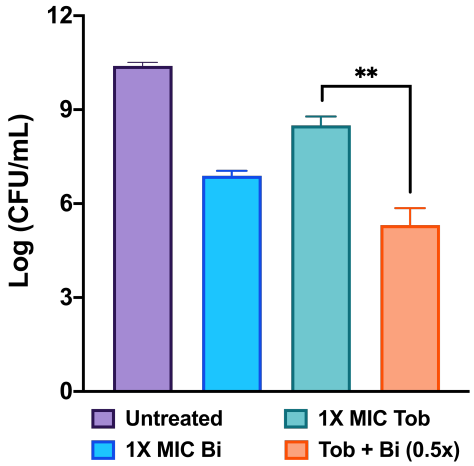

Figure S4. Biofilm killing assay of the combination of bismuth and tobramycin. The concentrations of bismuth and tobramycin as single drug were 25 μg/mL and 128 μM respectively. The concentrations of bismuth and tobramycin in the combination were 12.5 μg/mL and 64 μM respectively.

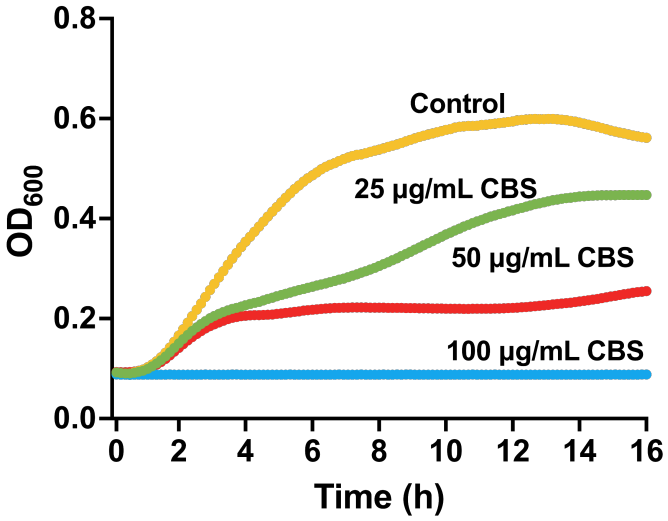

Figure S5. Growth curves of *B. cepacia* in the presence of different concentrations of CBS. The initial inoculum of bacteria was $1x10^8$ CFU/mL.

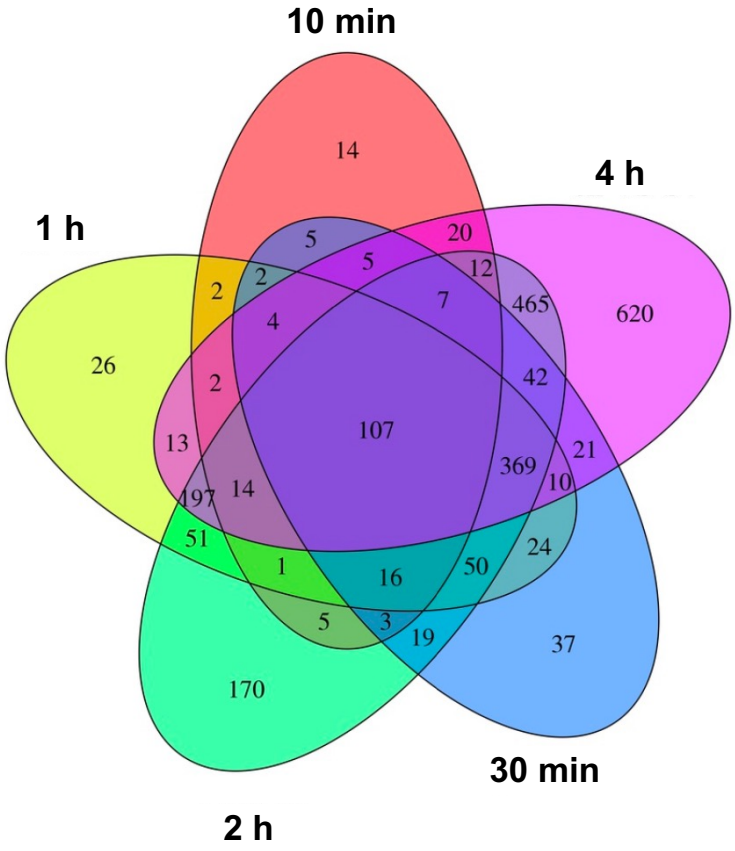

Figure S6. Venn diagram of the differentially expressed genes. The overlapping number stands for the mutual differentially expressed genes between the different comparisons and the non-overlapping numbers specify the genes unique to each condition.

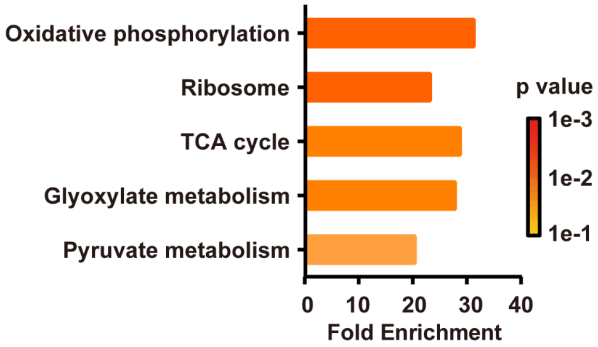

Figure S7. KEGG pathway enrichment revealing five pathways involving in Bi-binding proteins.

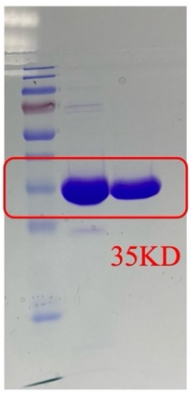

Figure S8. SDS gel showing the highly pure BcMDH. The MW of BcMDH is ~35KD.

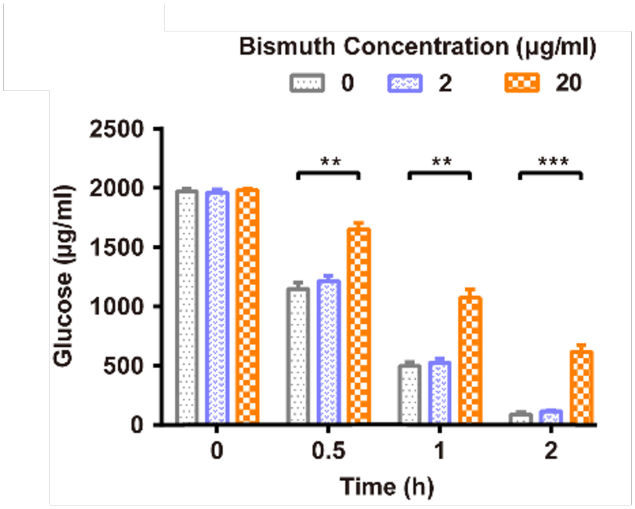

Figure S9. Glucose consumption rate of *B. cepacia* in the presence of different concentrations of bismuth.

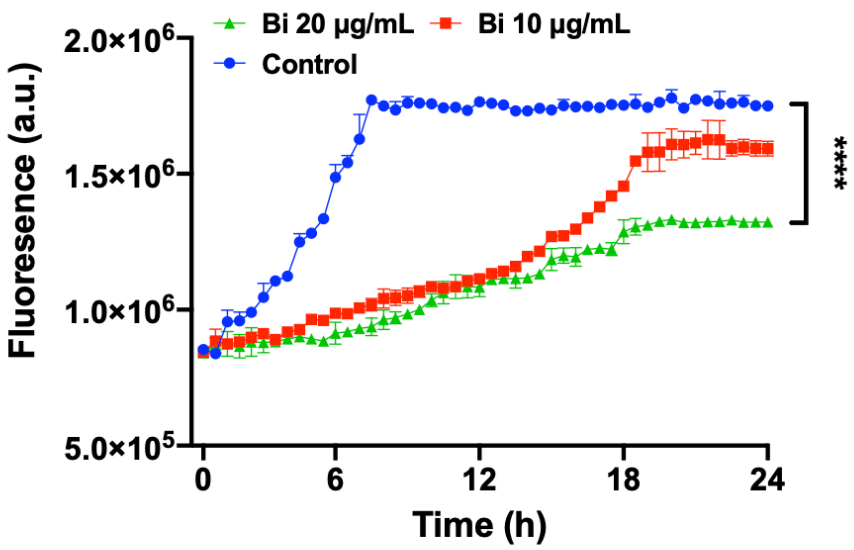

Figure S10. Oxygen consumption of *B. cepacia* in the presence of moderate (10 □g/ml) and subinhibitory (20 □g/ml) dosage of CBS.

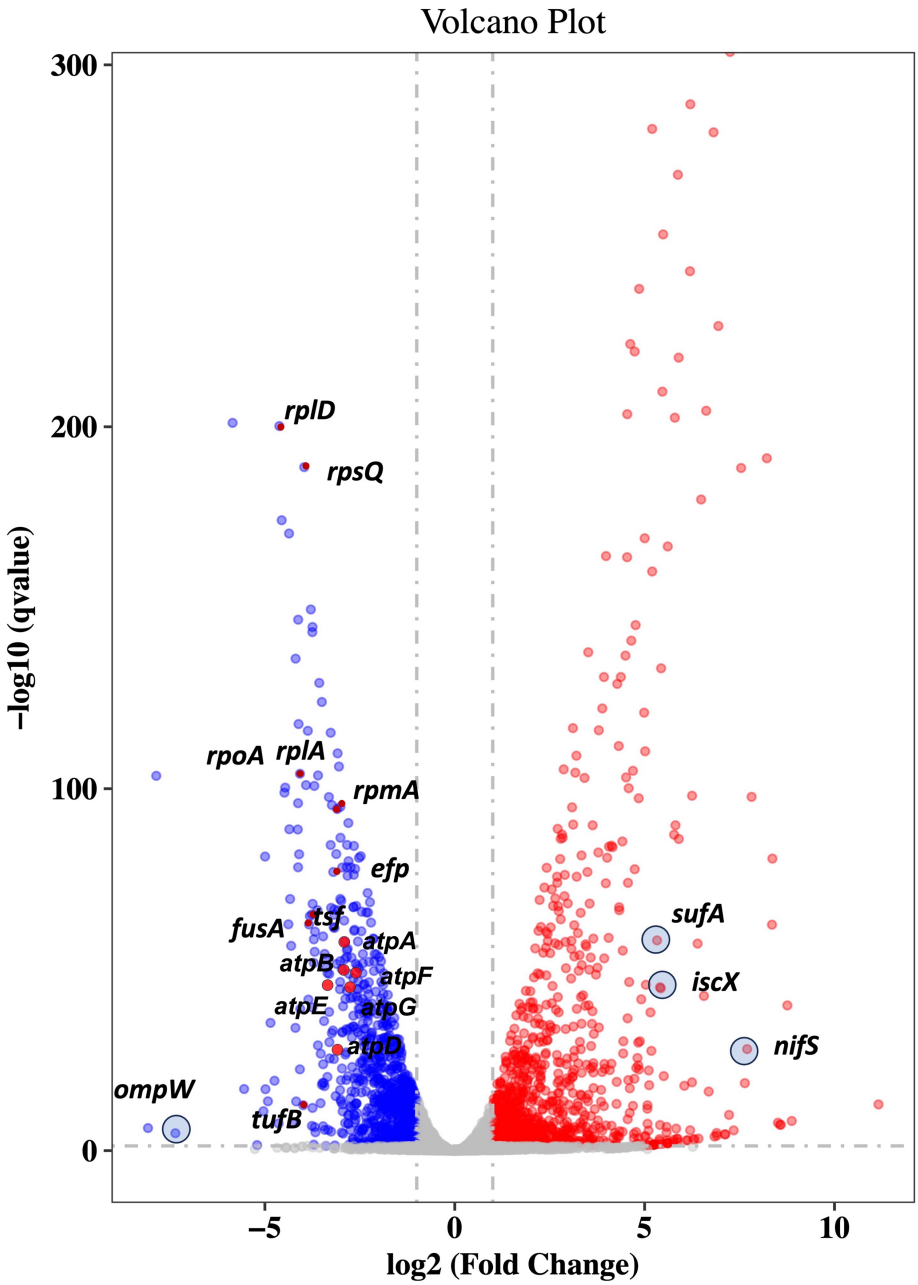

Figure S11. Volcano plot demonstrates differential expression genes (DEGs) of *B. cepacia* after 4 h CBS treatment. Note that those genes changed significantly are highlighted.

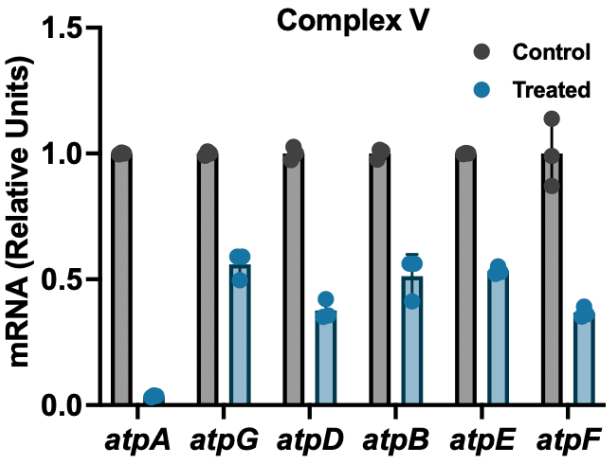

Figure S12. Analysis of nascent mRNA level of genes encoding ATPase of *B. cepacia* in the presence of CBS.

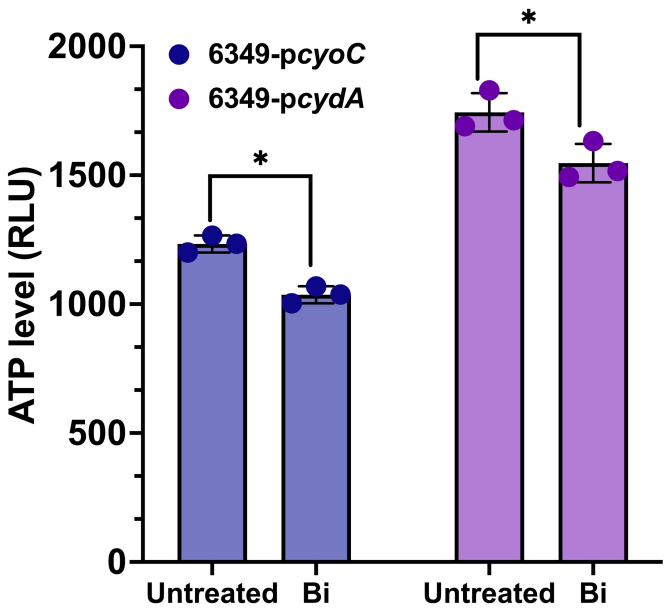

Figure S13. ATP production in *B. cepacia* variant strains in the presence of high dose of CBS (40 μg/mL).

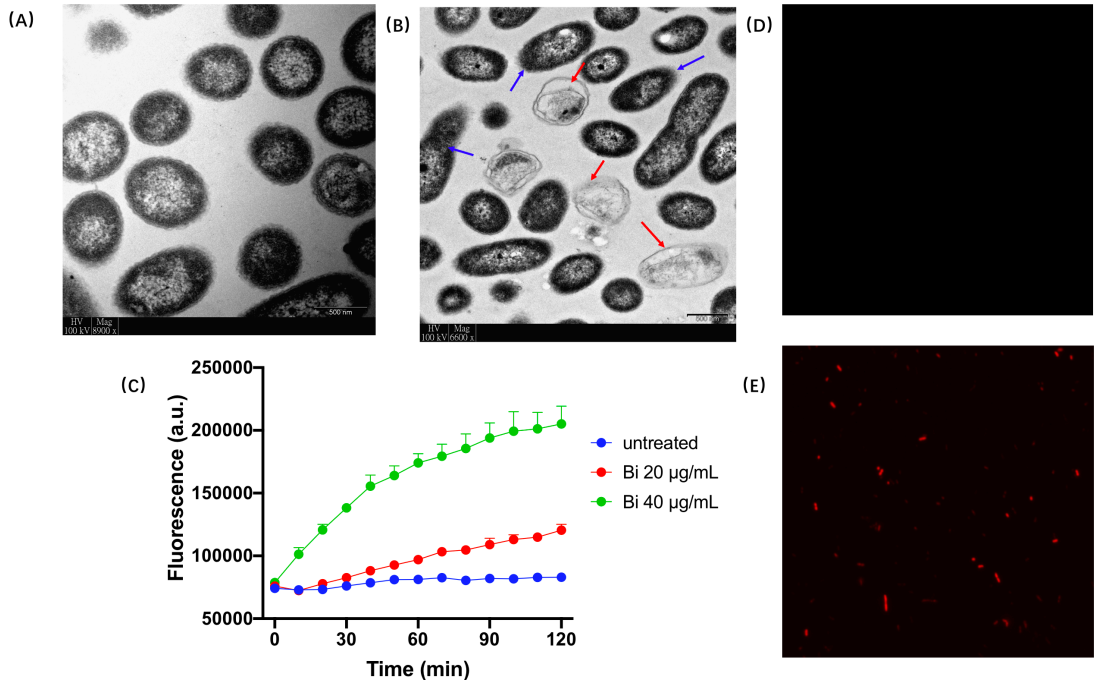

Figure S14. Bismuth-induced impaired membrane integrity of *B. cepacia*. (A) TEM image of untreated *B. cepacia*. (B) treatment of CBS at a subinhibitory concentration caused separation of outer membrane from cellular membrane (red arrow) and the leakage of cytoplasmic contents (blue arrow). (C) CBS dosage-related membrane permeability increment. (D) PI-staining of untreated *B. cepacia*. (E) PI-staining of *B. cepacia* with treatment of 40 μg/mL CBS.

Table. S1 Inhibitory zone diameters.

Compounds	**Average inhibition zone in diameter (mm)**					
	E. coli	***S. aureus***	***P. aeruginosa***	***B. cepacia***	***A. baumannii***	***K. pneumonia***
$Bi(NTA)_3$	<6	<6	<6	8	<6	<6
CBS	<6	<6	<6	20	<6	<6
RBC	<6	<6	<6	16	<6	<6
AgNPs	12	14	8	14	14	8

*RBC represents Ranitidine bismuth citrate.

Table. S2 MIC value of antibiotics against Bcc strains.

Antibiotics	**MIC (μg/mL)**		
	B. cenocepacia **J2315**	***B. cepacia*** **6349**	***B. multivorans*** **C1576**
Amoxicillin	>256	>256	>256
Tetracycline	32	32	32
Ciprofloxacin	4 (12 μM)	1 (3 μM)	2 (6 μM)
Metronidazole	>256	>256	>256
Levofloxacin	2 (6 μM)	1 (3 μM)	2 (6 μM)
Meropenem	16	2	8
Chloramphenicol	16	8	8

Table. S3 Cytotoxicity of bismuth compounds towards A549 cell.

Bismuth compounds	**CC_{50} (μM)**
$Bi(Hino)_3$	24.16
$Bi(NAC)_3$	>256
$Bi(GSH)_3$	>256
$Bi(Tro\text{-}NH_2)_3$	>256
Bi(DTT)OTf	>256
$Bi(p\text{-}PySH)_3(NO_3)_3$	>256
$Bi(TS)_3$	>256

Table. S4 Bi^{III} binding proteins of *B. cepacia* in membrane.

No.	Gene product	Protein Score	Protein Score C. I. %	Protein MW (KDa)
1	50S ribosomal protein L27	102	95.7	9.08
2	30S ribosomal protein S6	56	92	14.40
3	OmpA/MotB domain protein	353	100	21.62
4	Cytochrome bo ubiquinol oxidase subunit III	97	100	22.37
5	50S ribosomal protein L4	132	100	23.05
6	30S ribosomal protein S2	304	100	27.10
7	Competence protein ComL	88	100	30.70
8	ABC transporter/periplasmic glutamate/aspartate-binding protein	383	100	33.51
9	Outer membrane protein assembly factor	110	100	30.52
11	Cytochrome bd oxidase subunit I	327	100	58.85

Table. S5 Bi^{III} binding proteins of *B. cepacia* in cytosol.

No.	Gene product	Protein Score	Protein Score C. I. %	Protein MW (KDa)
1	Molecular chaperone GroES	211	100	10.47
2	Carboxypeptidase regulatory-like domain protein	121	100	15.17
3	Ribosome hibernation promoting factor	151	100	15.46
4	Universal stress protein UspA	301	100	16.97
5	Single-stranded DNA-binding protein	207	100	18.74
6	Hypothetical protein P350_38680	199	100	24.01
7	Orotate phosphoribosyltransferase	119	100	26.45
8	ABC transporter	308	100	32.72
9	Ketol-acid reductoisomerase	140	100	36.50
10	Ornithine decarboxylase	222	100	82.00
11	Deacylase/Lipid A deacylase	121	100	20.81
12	Malate dehydrogenase	197	100	84.93
13	Malate synthase	137	100	59.35
14	Succinate-CoA ligase, beta subunit	89.9	99.98	41.31
15	Alkyl hydroperoxide reductase	91	99.998	20.48

Table. S6 Primers used in this study.

Gene	Forward	Reverse
rpoS	GAACGCACGACATACTTGCC	GCGTGAAAACCTCGCTGAAG
gryB	CCACTGAGGCTTCGACTGAG	CGTGATGGGTACGTTCGACA
ompR	CGAACACTTCGTATTCGCGG	AGGAAATTCCGCTGACCACC
tsf	GTGATCGTCGAGTACACGGG	TTCCGTCTCGATCAGTTCCG
tufB	GTGCCGTGTTGATCGTGATG	CACGACGGTTCTGACGAAGA
NuoJ	GTGCTGGCATTCTTCAACGC	GCATCATCACGACGAACAGG
fusA	CGCGTTCGTCAACAAGATGG	ATCGGAATCTGGATCGGCAC
efp	GACCGCAAGGAAGTGACGTA	TCGAGGTAGTTCAGCGCTTC
ompW	CCATATCGCCACCGAGTTCA	GTACTTGAGGAGGAGCGCAG
AtpF	GAATCGACTGGTCCACGCTC	GCGATGATGTCGCTGACCG
AtpE	GCAAGTACATCGAAGCCTGC	AACAGCATTGCGACACCAAC
AtpB	ATCCGTCCGAGTACATTGCG	CCGCATACGATCGACCAGAA
AtpD	TCTACGACGCGCTCATTCTC	CCGTGTTCTTCACGGTCAGA
AtpG	CTTACACGCGCTTCGTCAAC	CGGCTCGTAGATGTAGTCCC
AtpA	GAAGTTCACGAACGGCGAAG	ATCGAGATCACGTTCGTCGG
rplD	CCAGGTACAGGTTCTCGTCG	AGGACATCATCCTCGAAGCG
RplA	ATGCGAAGAAGTCGGACCAG	CCAGGTCTTCCATACCGACG
rpoA	AGCTGCATAACCGTGACGAA	TCGGGTTGATGACTTCGCAA

Table. S8 Strains and plasmids used and constructed in this study.

Strain	Application
B. cepacia 6349	Susceptibility test
B. cenocepacia J2315	
B. multivorans C1567	
E. coli BL21	Protein purification
E. coli S17	Plasmid transformation/conjugation
B. cepacia 6349-p*cyoC*	Target validation/overexpression strain
B. cepacia 6349-p*cydA*	
B. cepacia GFP	

Plasmid	Application
pHisSUMO	Protein expression
pHisSUMO-*mdh*	
pMLBAD	Gene overexpression
pMLBAD-*cyoC*	
pMLBAD-c*ydA*	
pMLS7-GFP	
pMLS7	

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