Supplementary Information

Specific inactivation of an anti-fungal bacterial siderophore by a fungal plant pathogen

Running title: Fungal transformation of pyochelin

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Supplementary Material and Methods

Cultivation of strains and media

Burkholderia cenocepacia 869T2 was collected by Prof. Chieh-Chen Huang's Lab (National Chung Hsing University, Taiwan) and maintained in the 869 broth medium. *Pseudomonas aeruginosa* ATCC 15692 (strain PAO1) *and P. protegens* NRRL B-23932 (strain Pf-5) were purchased from American Type Culture Collection (ATCC) and Agricultural Research Service Culture Collection (NRRL-Northern Regional Research Laboratory) and maintained in Lysogeny-broth (LB) broth medium. *Phellinus noxius* strain 2252 was collected by Prof. Chia-Lin Chung's Lab (National Taiwan University, Taiwan) [1] and maintained in potato dextrose agar (PDA) agar medium. For siderophore production, *P. aeruginosa* PAO1, *P. protegens* Pf-5, and *B. cenocepacia* 869T2 were cultured in the GGP broth medium. All the strains were cultured at 30 ˚C.

869 broth medium: 10 g tryptone (BD Bacto), 5 g yeast extract (Merck), 5 g NaCl (FluKa), 1 g D-glucose (Merck), and 0.345 g $CaCl_2 \cdot 2\ H_2O$ (J.T.Baker) (pH 7) in one liter of H_2O.

LB broth medium (BD Difco): 10 g tryptone, 5 g yeast extract, and 10 g NaCl (pH 7) in one liter of H_2O.

PDA medium (BD Difco): 4 g potato extract, 20 g dextrose, and 15 g agar in one liter of H_2O.

GGP broth medium: 30 mL glycerol (Merck), 10 g proteose peptone (Oxiod), and 0.5 g each of K_2HPO_4 (J.T.Baker) and $MgSO_4$ (J.T.Baker) in one liter of distilled water [2].

MALDI-TOF IMS analysis

B. cenocepacia 869T2 was firstly incubated in 869 medium to stationary-phase (OD600 = 1.5-2) then transplanted to a PDA plate as a spot (10 μL). An 8-mm mycelial plug of *P. noxius* from a 7-day fungal plate was placed at the opposite edge of the bacterial spot at a 1.5-cm distance. The dual-culture samples were incubated at 30 ˚C for four days for monitoring the metabolic changes by using MALDI-TOF IMS.

On the other hand, we prepared samples for monitoring the biotransformation of pyochelin and *ent*-pyochelin by *P. noxius*. 15 μL of crude bacterial extract (total 1 mg) or pyochelin (0.1 mg) was added to a sterile 8-mm sterilized paper disk on a PDA plate. Then, an 8-mm fungal mycelial plug was placed at the opposite edge of the paper disc at a 1.5-cm distance for a further 4-8 hours incubation.

The sample areas of agar media containing the bacterial colonies (or paper disks) and fungal mycelia were excised from the Petri dish and transferred onto the MALDI stainless steel target plate. A 1:1 mixture of α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid (universal matrix, Sigma-Aldrich) was spread on top of the sample using HTX TM-Sprayer (HTX Tech., Chapel Hill, NC, USA). The parameters of HTX TM-Sprayer were: 1200 mm/min velocity, 0.1 mL/min flow

rate, 12 passes, 3 mm line spacing, and a nozzle temperature of 80 ˚C. Once the sample was covered entirely with matrices, it was exposed in a 37 ˚C incubator overnight until it was deemed dried. The IMS data were collected using a Bruker Autoflex Speed MALDI-TOF/TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The settings of the mass spectrometer were: 1000 shots/raster, 19.0 and 16.6 kV for the ion sources 1 and 2, 8.7 kV for the lens, 21 and 9.4 kV for reflectors 1 and 2, respectively. We selected mass range from *m*/*z* 100-2000 with 25% laser power, positive and reflectron mode, and the raster was set to 800 μm. We used the universal matrix and peptide standard I (Bruker Daltonik GmbH, Bremen, Germany) for MS calibration. All IMS data were processed with TIC normalization mode and analyzed using Fleximaging 3.3 (Bruker Daltonik GmbH, Bremen, Germany).

UPLC/ESI-Qrbitrap MS and data analysis

The cultural agar plates were extracted by ethyl acetate then dried completely under vacuum. 10 L of extracts (10 mg/mL in methanol) were subjected to LC-MS analysis. The high-resolution LC-MS and tandem mass data were collected by a Thermo UPLC-ESI Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with the following settings: ACQUITY UPLC BEH C18 column (Waters, Milford, MA, USA), 1.7 μm, 2.1 × 100 mm; flow rate: 0.4 mL/min; 0-6 min 5% to 99.5% ACN, 6-8 min 99.5% ACN, 8-8.2 min 99.5% to 5% ACN, 8.2-10 min 5% ACN, both ACN and H_2O contain 0.1% formic acid; mass range between 50-1500 Da; positive ion mode). The mass data (.RAW) were converted to mzXML file format and submitted to XCMS [3] (https://xcmsonline.scripps.edu) and GNPS [4] (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp) to generate chemical profiling and molecular networking and the molecular networking data were visualized in Cytoscape 3.7.1. Data

Construction of pyochelin biosynthetic gene-null mutant strains

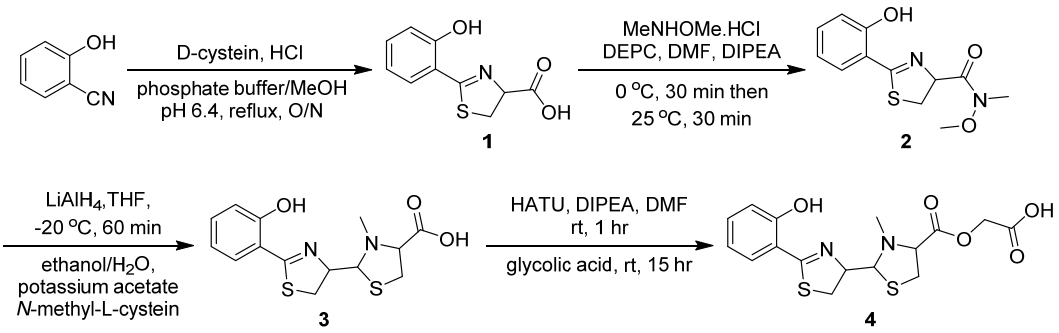
We constructed the PCM184-Δ*pchF* for making insertion mutation of pyochelin biosynthetic genes *pchF* in *B. cenocepacia* 869T2. The pCM184 was purchased from Addgene. The regions for homologous recombination in *B cenocepacia* 869T2 were amplified by primer sets (a list of primer sets was shown below, Genomics, Taiwan). The PCR products were incorporated into the yT&A vector according to the instruction (Yeastern Biotech). The vectors were digested with EcoRI (NEB) and KpnI (NEB), and pyochelin biosynthetic gene *pchF* fragments were cloned into the EcoRI /KpnI site of the digested pCM184, followed by transforming into the *E. coli* S17-1 λ *pir*. The *E. coli* S17-1 was applied to transform PCM184-Δ*pchF* into *B. cenocepacia* 869T2 in this work. *B. cenocepacia* 869T2 and *E. coli* S17-1 were cultured for 16 hours in LB at 30 and 37 ˚C, respectively, and both were washed by LB twice. The pellets were suspended by LB again. The optical density of

donor and recipient cells was OD_{600} = 1.0, and the mixtures contained 1:1, 1:10, and 10:1 (v:v) of two cells, and were spotted 10 μL on LB agar plates and cultured at 30 ˚C for 16 hours. The colonies were scraped up and spread on LB agar plates with gentamycin (10 μg/mL, Sigma-Aldrich) and kanamycin (400 μg/mL, Sigma-Aldrich), and then the 869T2 conjugants were picked and checked by PCR after 48 hours. The conjugants only could grow when the plasmid was integrated into the 869T2 genomic DNA. The insertion sites were confirmed by using PCR, and check primer sets were shown below.

List of primer sets used in this study

Vector construction	Sequence (5'->3')
PchF-36-F	CGGCAGATCGAATTGTGGTGC
PchF-648-Kpn-R	GGTACCCTACCATTCGGCAAGCAGCAGTTG
Mutant examination	**Sequence (5'->3')**
pchF-ck1-F	CGACGACGTACTCGATCTCTG
pchF-ck2-R	GTCTGGCTGATCATGTAGCG
pCM184-pchF-ck3-R	GAGCAAGACGTTTCCCGTTG
pCM184-pchF-ck4-F	AGCGTTTCTGGGTGAGCAAA

Synthesis of pyochelin and pyochelin-GA



Pyochelin was synthesized followed the procedures by Ankenbauer et al. [5]. 2-Hydroxy-2-benzonitrile (1.0 g, 8.4 mmol, Sigma-Aldrich), D-cysteine hydrochloride (COMBI-BLOCKS) was dissolved in methanol (32.5 mL, Sigma-Aldrich) and phosphate buffer 0.1 M pH 6.4 (32.5 mL). The solution was adjusted to pH 6~7 by adding solid K_2CO_3 (Sigma-Aldrich) and stirred overnight at 60˚C. The crude material was concentrated under reduced pressure and diluted with water (50 mL) and adjusted to pH 2.0 by adding solid citric acid (Sigma-Aldrich). The solution was extracted with CH_2Cl_2 (3 x 50 mL, Sigma-Aldrich) to give the organic layers, further

dried over Na_2SO_4 (Sigma-Aldrich) and filtered. Removed solvent under reduced pressure to provide the thiazoline compound (**1**, 1.8 g, 8.0 mmol, 96 %).

N,O-dimethylhydroxylamine hydrochloride (0.5 g, 5.5 mmol, Sigma-Aldrich) and diethylcyanophosphonate (DEPC, 1.0 g, 5.5 mmol, Sigma-Aldrich) in DMF (7.5 mL, Sigma-Aldrich) at 0 ˚C and diisopropylethylamine (DIPEA, 1.9 ml, 5.5 mmol, Sigma-Aldrich) were added into a solution of thiazoline (**1**, 1.1 g, 5 mmol). The mixture was stirred for 30 min at 0 ˚C, then another 30 min at room temperature. 3% solution of citric acid (45 mL) was added, and the precipitate was conserved. The filtrate was extracted twice with a mixture of toluene (Sigma-Aldrich) and ethyl acetate (1:1, v/v, Sigma-Aldrich). The organic phase was washed with water and evaporated the organic phase. The residue and the former precipitate were pooled and dried under reduced pressure to yield a yellow powder (**2**, 1.2 g, 4.6 mmol, 84 %), which was recrystallized from methylene chloride or purified by using silica gel chromatography with hexane and ethyl acetate.

Weinreb amide hydroxamate (**2**, 250 mg, 0.94 mmol) and lithium aluminum hydride (107 mg, 2.82 mmol, Sigma-Aldrich) were mixed in anhydrous THF (20 mL, Sigma-Aldrich) and cooled down to -30 ˚C for 60 min. Then a saturated aqueous solution of ammonium chloride (10 mL) and a final 5% (v/v) sulfuric acid (5 mL, Sigma-Aldrich) were successively added. The organic phase was conserved, and the aqueous phase was further extracted with ethyl acetate (3 x 10 mL). The organic phases were pooled and evaporated under reduced pressure to give crude 2'-(2-hydroxyphenyl)-2'-thiazoline-4'- carboxaldehyde.

2'-(2-Hydroxyphenyl)-2'-thiazoline-4'-carboxaldehyde (106.3 mg, 0.513 mmol) was dissolved in a mixture of ethanol (16 mL) and water (4 mL), followed by the addition of potassium acetate (279.7 mg, 3.591 mmol, Sigma-Aldrich) and *N*-methylcysteine (140.8 mg, 1.026 mmol, Toronto Research Chemicals). After 24 h, removed the EtOH under reduced pressure, and water (50 mL) was added. The aqueous phase was acidified to pH 5.0, then partitioned with ethyl acetate (3 x 100 mL). The crude pyochelin mixture was obtained from the ethyl acetate layer and purified by HPLC to give pure pyochelin (**3**, 160.5 mg, 0.495 mmol, 96.5 %).

The crude pyochelin (30 mg, 0.09 mmol), HATU (35.4 mg, 0.09 mmol, Sigma-Aldrich), and DIPEA (30 μL, 0.18 mmol, Sigma-Aldrich) were added to anhydrous DMF (4 mL, Sigma-Aldrich) under argon. After stirring for 1 hour, glycolic acid (10.6 mg, 0.14 mmol, Sigma-Aldrich) and DIPEA (15 μL, 0.09 mmol Sigma-Aldrich) in anhydrous DMF was added. The resulting mixture was stirred for 15 hours under dark, diluted with CH_2Cl_2, and washed with water (2 x 100 mL). The organic layer was dried over Na_2SO_4 (Sigma-Aldrich) and evaporated. The residue was further purified by HPLC to give pyochelin-GA (**4**, 3.5 mg yield 9.9 %).

Compound **1**, HRMS $[M+H]^+$: 224.0367 (calculated for $C_{10}H_{10}NO_3S$, 224.0381).
^1H NMR [500 MHz, $(CD_3)_2CO$] δ 7.47 (dd, *J* = 7.8, 1.7 Hz,1H), 7.43 (td, *J* = 7.8, 1.7 Hz, 1H), 6.98 (dd, *J* = 8.4, 0.98 Hz, 1H), 6.94 (td, *J* = 8.4, 0.98 Hz, 1H), 5.54 (dd, *J* = 9.3, 7.5 Hz, 1H), 3.79 (dd, *J* = 10.9, 9.3, 1H), 3.75 (dd, *J* = 11.3, 7.5, 1H). ^{13}C NMR [125 MHz, $(CD_3)_2CO$] δ 174.6, 171.4, 160.2, 134.5, 131.6, 120.0, 118.0, 116.9, 77.6, 32.2.

Compound **2**, HRMS $[M+H]^+$: 267.0793 (calculated for $C_{12}H_{15}N_2O_3S$, 267.0803).
^1H NMR [500 MHz, $(CD_3)_2CO$)] δ 7.45 (d, *J* = 8.1 Hz, 1H), 7.42 (t, *J* = 8.1 Hz, 1H), 6.92-6.97 (m, 2H), 5.83 (t, *J* = 8.5 Hz, 1H), 3.88 (s, 3H), 3.69 (m, 2H), 3.25 (s, 3H). ^{13}C NMR [125 MHz, $(CD_3)_2CO$)] δ 174.3, 170.4, 160.0 134.2, 131.5, 119.8, 117.8, 116.9, 75.6, 62.1, 33.6, 32.7.

Compound **3**, HRMS $[M+H]^+$: 325.0664 (calculated for $C_{14}H_{17}N_2O_3S_2$, 325.0675).
^1H NMR [500 MHz, $CDCl_3$] δ 7.53 (t, *J* = 7.4 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 6.92 (t, *J* = 7.4 Hz, 1H), 4.75 (m, 1H), 4.32 (d, *J* = 9.4 Hz, 1H), 3.96 (t, *J* = 8.0 Hz, 1H), 3.82 (dd, *J* = 8.9, 11.8 Hz, 1H), 3.5 (dd, *J* = 8.9, 12.0 Hz, 1H), 3.53 (*J* = 8.0, 12.0 Hz, 1H), 3.48 (*J* = 8.0, 12.0 Hz, 1H), 3.32 (dd, *J* = 4.6, 11.9 Hz, 1H), 2.63 (s, 3H). ^{13}C NMR [125 MHz, $CDCl_3$] δ 181.3, 174.6, 160.7, 138.6, 132.0, 120.3, 118.7, 112.2, 78.5, 72.8, 72.1, 45.7, 34.6, 33.0.

Compound **4**, HRMS $[M+H]^+$: 383.0735 (calculated for $C_{16}H_{19}N_2O_5S_2$, 383.0729).
^1H NMR [600 MHz, $(CD_3)_2CO$] δ 7.39-7.45 (m, 2H), 6.92-6.97 (m, 2H), 5.29 (dt, *J* = 4.8, 9.0 Hz, 1H), , 4.73 (d, *J* = 5.8 Hz, 1H), 4.68 (d, *J* = 4.8 Hz, 1H), 3.77 (dd, *J* = 6.4, 9.2 Hz, 1H), 3.55 (dd, *J* = 9.0, 11.1 Hz, 1H), 3.47 (dd, *J* = 9.0, 11.1 Hz, 1H), 3.17-3.23 (m, 2H), 2.64 (s, 3H). ^{13}C NMR [150 MHz, $(CD_3)_2CO$)] δ 173.2, 171.1, 168.9, 159.8, 134.1, 131.4, 119.8, 117.7, 117.0, 80.0, 76.5, 72.9, 61.5, 40.5, 33.1, 32.4.

Chrome Azurol S (CAS) assay

Pyochelin and pyochelin-GA were evaluated for siderophore efficiency units by universal CAS assay. CAS reagent was prepared as a description of Schwyn and Neilands (1987) [6]. 60.5 mg CAS was dissolved in 50 mL distilled water and added 10 mL of 1 mM $FeCl_3 \cdot 6H_2O$ (J.T.Baker) prepared in 10 mM HCl (CAS-iron solution). Hexadecyl trimethyl ammonium bromide (HDTMA, Sigma-Aldrich) solution was prepared by mixing 72.9 mg HDTMA in 40 mL distilled water. CAS-iron solution was added to the HDTMA solution under stirring and sterilized by autoclave. PIPES sodium salt solution was prepared by mixing 30.24 g PIPES (Sigma-Aldrich) in 900 mL distilled water and then sterilized. The CAS-HDTMA and PIPES solution were mixed right before use as CAS reagent. Pyochelin and pyochelin-GA were dissolved in methanol and added 10 μL in

190 μL CAS reagent to give the final concentration of 2.5, 1.25, 0.63, 0.31, and 0.16 mM. The reference was prepared by adding 10 μL of methanol in 190 μL CAS reagent. The reactions were incubated at 30 ˚C, and the 630 nm absorbances of tested samples and reference were measured at 3 and 24 hours, three replicates for each sample. The siderophore unit was calculated according to the following formula: (Ar-As)/Ar × 100% (Ar = absorbance of the reference and As = absorbance of the tested sample).

Molecular Modeling of pyochelin and pyochelin-GA with FptA receptor

All procedures were performed in Maestro version 11.5 (Release 2018-1, Schrödinger, LLC, New York, NY). The stereochemistry of pyochelin (*4'R,2''R,4''R*) and pyochelin-GA (*4'R,2''R,4''R*) used in this study are the same configuration as the ligand in Fe^{3+}-pyochelin-FptA co-crystal structure (PDB code: 1XKW [7]) for bioactive conformation. Both ligand structure files were first built by Chem3D Pro 12.0 software (CambridgeSoft, Cambridge, MA, USA) and refined by the LigPrep [8] module of Maestro using the OPLS3 force field to generate ionization states for the 3D molecular structures.

The FptA receptor was refined using the Protein Preparation Wizard module of Maestro to remove water molecules and add hydrogens. The grid-enclosing box (10Å ^3 cube) was placed on the centroid of the ligand binding site, and the glide module was used to dock the compounds using the Extra Precision mode and the default parameters [9, 10].

The post docking rescoring was processed using the Prime MM-GBSA module of Maestro [11] with VSGB as solvent.

***In silico* structure modeling of Fe^{3+}-pyochelin and Fe^{3+}-pyochelinGA complex**

Density functional theory (DFT) and Solvent model density (SMD) calculations were used for modeling metal complex by the Gaussian 16 program package [12]. The optimization of structures were evaluated at the B3LYP/6-31+G(3d,p) level of theory with a modified script from Kircheva and Dudev [13].

Reactive oxygen species (ROS) in *P. noxius* 2252

P. noxius 2252 mycelia cultivated for two days on PDA was used to identify the ROS formation in disc diffusion assay with different drugs. Detection of ROS in the hyphae of *P. noxius* 2252 was performed using 2′,7′-dichlorofluorescin diacetate (DCFDA, Sigma-Aldrich D6883)[14]. In brief, different doses of pyochelin (PCH), pyochelin-GA (PCH-GA), 2,2′-bipyridyl (BIP, Sigma-Aldrich D216305), and deferoxamine (DFO, Sigma-Aldrich D9533) were added to 6-mm sterilized paper disk. Then, the dried paper disks were placed at the edge of the mycelia with 0.2 cm distance for a

further 4 hours incubation at 30 ˚C. After that, the hyphae near to the disk site were collected by using scotch tape. Next, a 10 µM DCFDA solution was dropped onto an individual glass slide for staining. Afterward, the samples adhered to scotch tape were placed face down on the slides to make them fully contact with the DCFDA fluid, incubating at 30 ˚C for 15 minutes in the dark. Subsequently, the samples were washed with phosphate-buffered saline (1x PBS, pH 7.4). Finally, the ROS in the hyphae from each sample was visualized using Carl Zeiss Axiovert 200M microscopy with an excitation wavelength of 504 nm and an emission wavelength of 529 nm. The densitometric mean fluorescence intensity of each treatment was quantified using AxiolVision Rel 4.8 with three biological replicates. The densitometric mean fluorescence intensity ratio was calculated using the following equation: densitometric mean fluorescence intensity ratio of DCFDA = treated (densitometric mean fluorescence intensity of DCFDA) / control (densitometric mean fluorescence intensity of DCFDA). Statistical analyses were performed using GraphPad Prism 8 for Windows. One-way analysis of variance (ANOVA) followed by the Newman-Keuls test was used to determine statistical differences ($p < 0.05$) throughout the study.

Antifungal assay of pyochelin and pyochelin-GA on *P. noxius* 2252

Potato dextrose agar (PDA) with the addition of $FeCl_3$ (Sigma-Aldrich, 157740) [15] or deferoxamine (DFO, Sigma-Aldrich D9533) was used for mimicking iron-rich or iron-deficiency conditions. Eight-millimeter mycelial plugs of *P. noxius* 2252 mycelia cultured for 4-5 days on PDA were transferred to PDA, PDA+$FeCl_3$, and PDA+DFO conditions then incubated at 30 ˚C for two days. Subsequently, *P. noxius* 2252 cultured on different conditions were used to evaluate the antifungal activity of pyochelin and pyochelin-GA using the disk-diffusion method at 30 ˚C for 24 hours. The antifungal assay was performed in two biological replicates.

Supplementary Figures

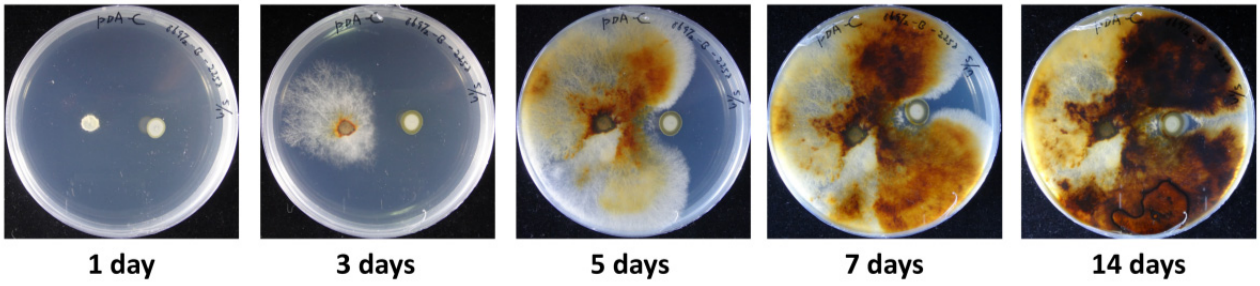


Fig. S1. The antagonistic assay of *B. cenocepacia* 869T2 vs. *P. noxius* 2252 on PDA at 30 ˚C.

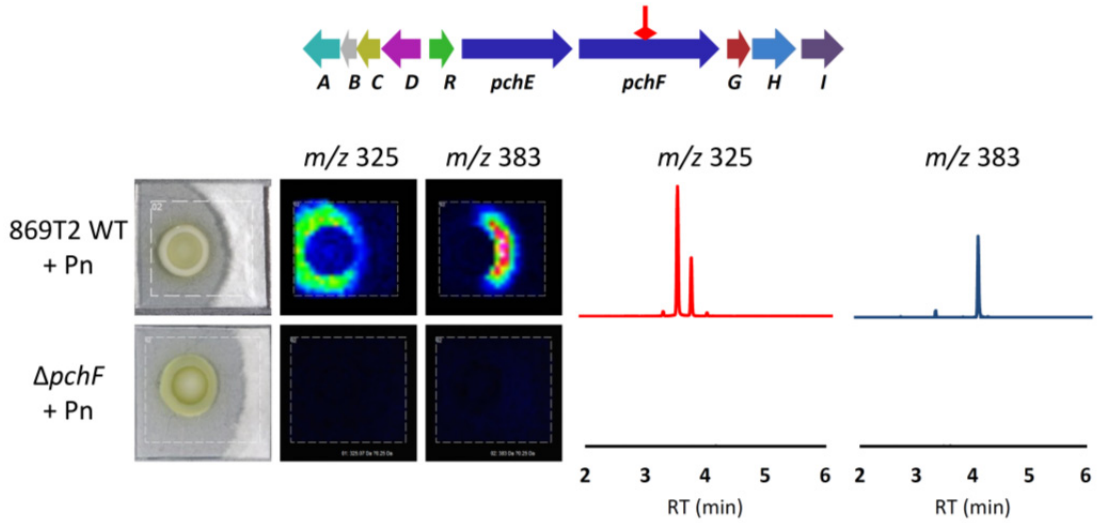


Fig. S2. MALDI-TOF IMS and LCMS analysis of *P. noxius* 2252 (Pn) dual-cultured with *B. cenocepacia* 869T2 (869T2 WT) and Δ*pchF* at 30 ˚C for four days. The biosynthetic gene cluster of pyochelin in *B. cenocepacia* 869T2 genome was shown, and the insertion disruption was applied to generate the *pchF* null mutant Δ*pchF*. The distributions of pyochelin (*m/z* 325) and pyochelin-GA (*m/z* 383) in the dual-culture condition were revealed by MALDI-TOF IMS (shown in the gradient heat map). The extracted ion chromatograms of pyochelin (*m/z* 325) and pyochelin-GA (*m/z* 383) were shown in red and blue. Neither pyochelin (*m/z* 325) or pyochelin-GA (*m/z* 383) was detected in the dual-culture sample of *P. noxius* 2252 vs. Δ*pchF*.

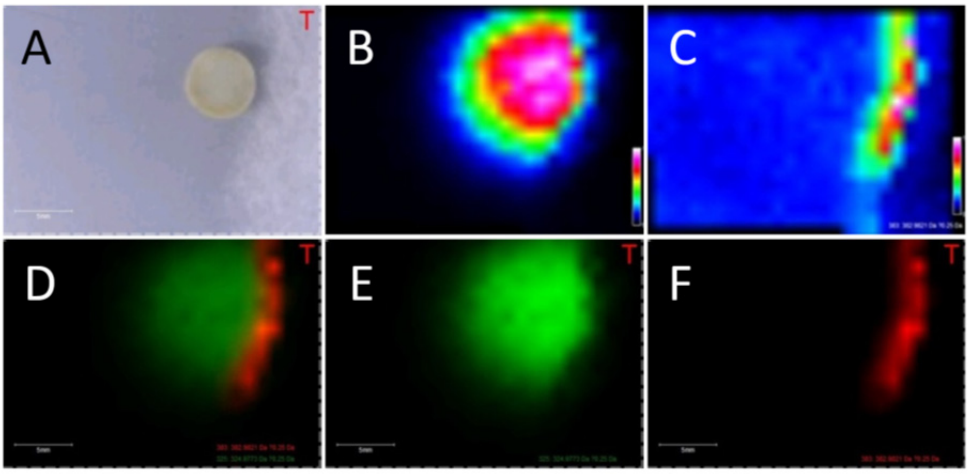


Fig. S3. MALDI-TOF IMS analysis of *P. noxius* 2252 treated with the crude extract of *B. cenocepacia* 869T2. (A) The crude extract was prepared in a paper disc placed nearby the mycelia of *P. noxius* 2252 for 4 hours. (B) Distribution of pyochelin (*m/z* 325) shown in the gradient heat map. (C) Distribution of pyochelin-GA (*m/z* 383) shown in the gradient heat map. (D, E, and F) Distributions of pyochelin (green) and pyochelin-GA (red).

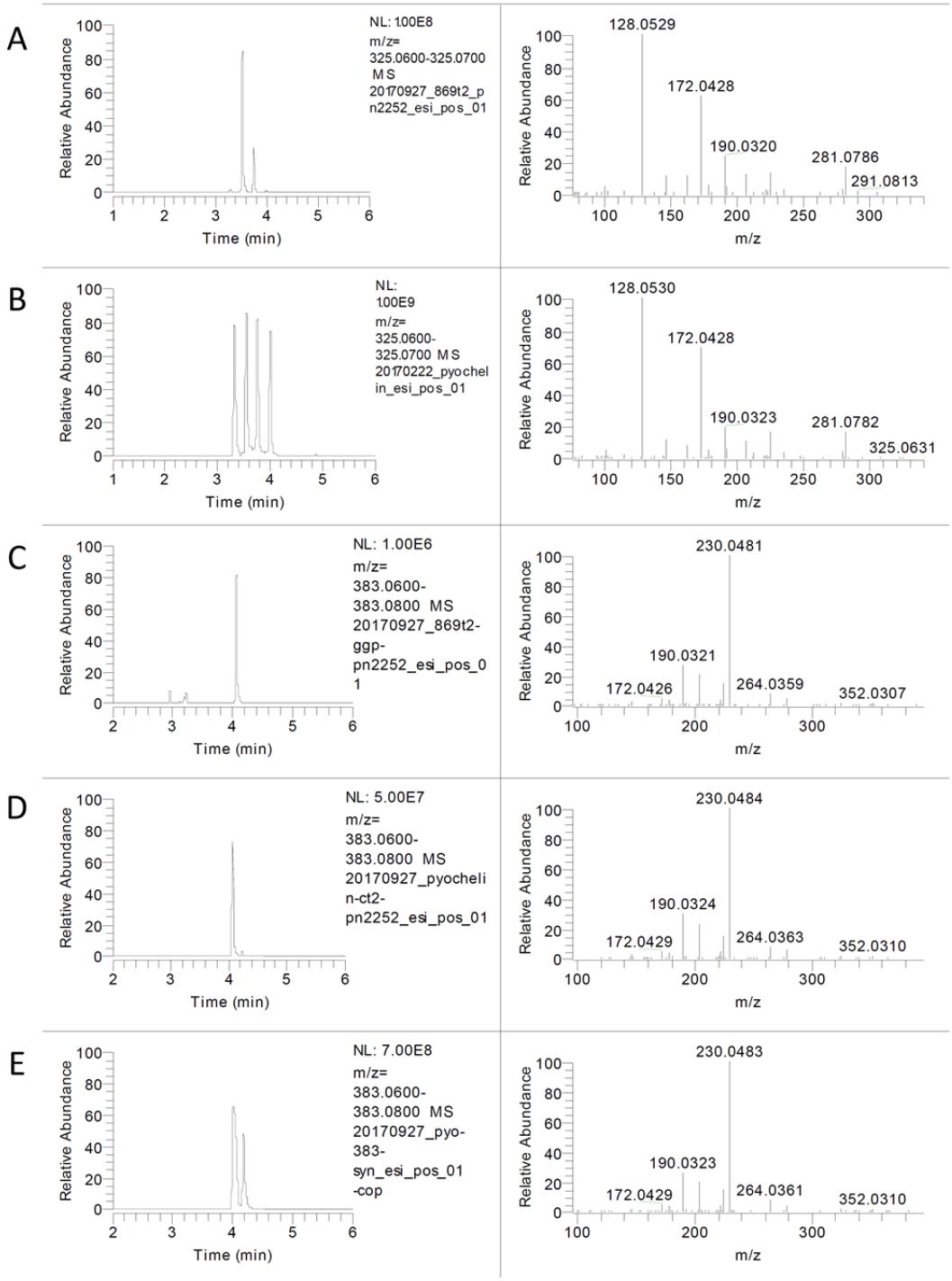


Fig. S4. LCMS analysis of pyochelin and pyochelin-GA. (A) The extracted ion chromatogram (EIC) and MS2 spectrum of pyochelin from the crude extract of *B. cenocepacia* 869T2 dual-cultured with *P. noxius* 2252 at 30 ˚C for two days. (B) The EIC and MS2 spectrum of synthetic pyochelin. (C) The EIC and MS2 spectrum of pyochelin-GA from the crude extract of *B. cenocepacia* 869T2 dual-cultured with *P. noxius* 2252 at 30 ˚C for two days. (D) The EIC and MS2 spectrum of pyochelin-GA from the crude extract of *P. noxius* 2252 treated with synthetic pyochelin at 30 ˚C for 8 hours. (E) The EIC and MS2 spectrum of synthetic pyochelin-GA.

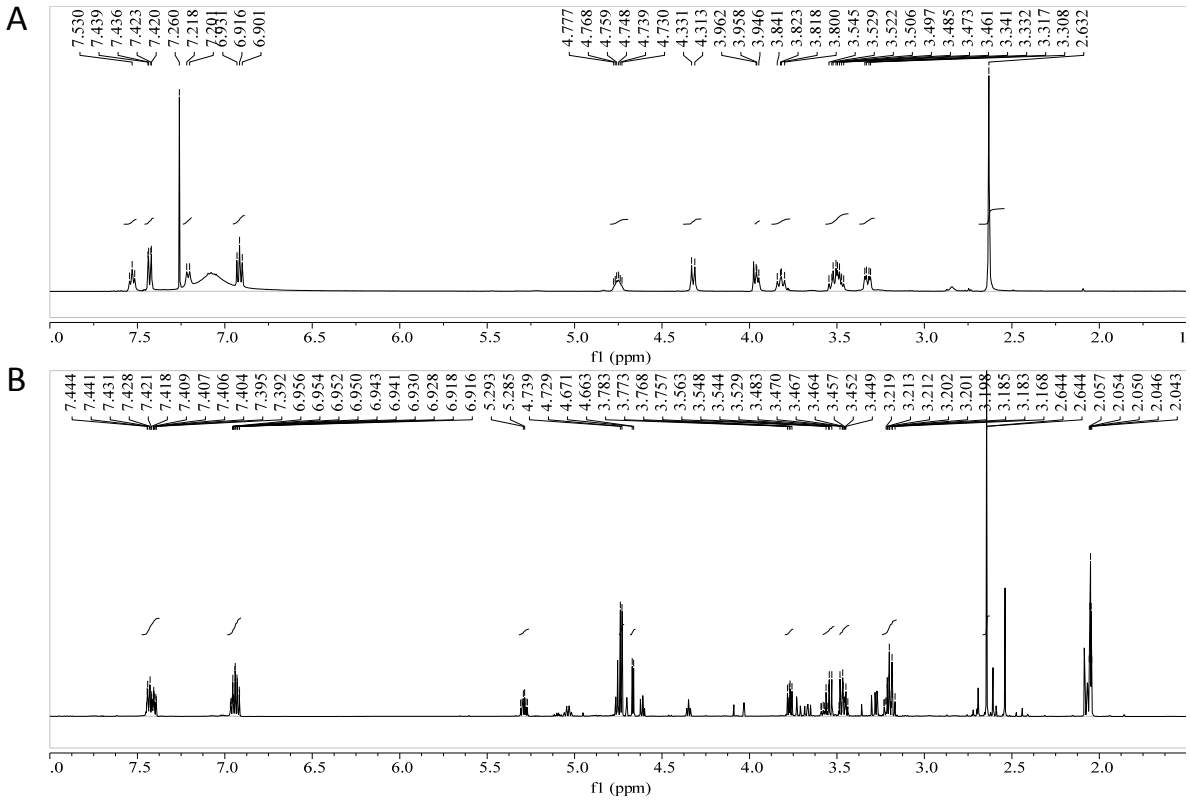


Fig. S5. ^{1}H NMR spectra of pyochelin (A, 500 MHz, $CDCl_3$) and pyochelin-GA (B, 600 MHz, $(CD_3)_2CO$).

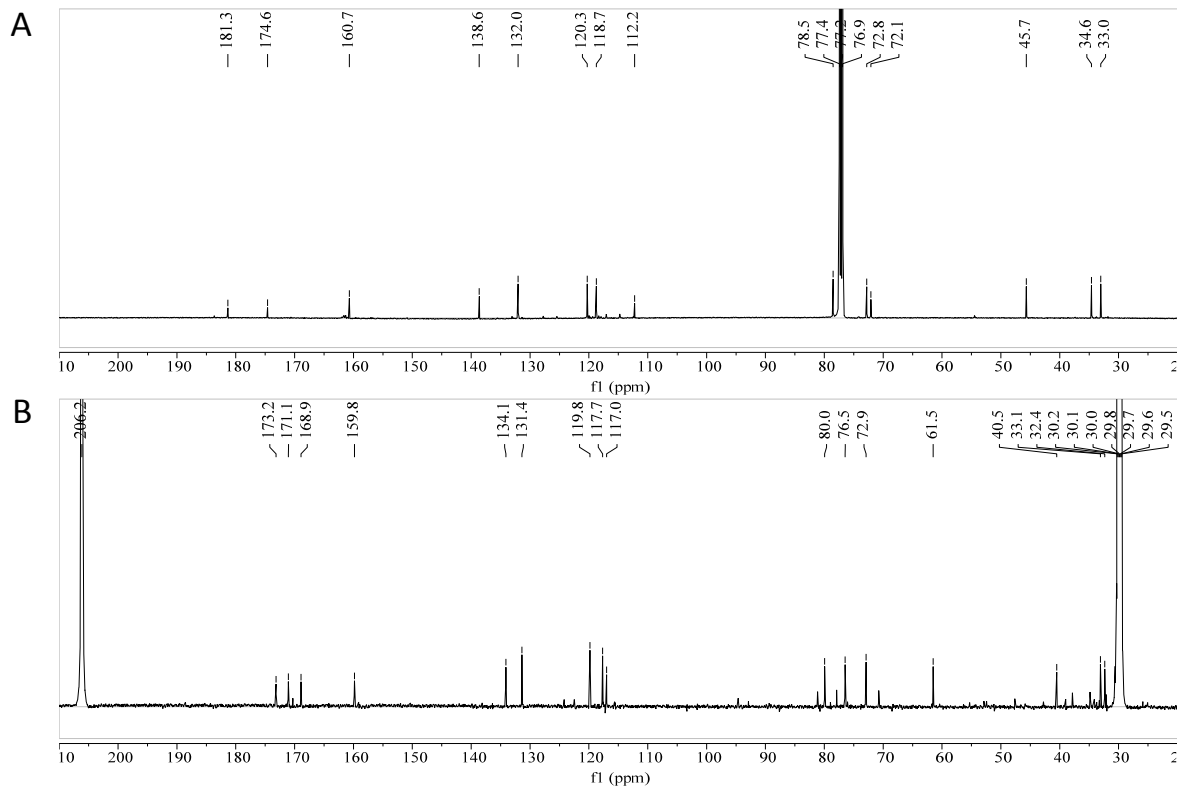


Fig. S6. ^{13}C NMR spectra of pyochelin (A, 125 MHz, $CDCl_3$) and pyochelin-GA (B, 150 MHz, $(CD_3)_2CO$).

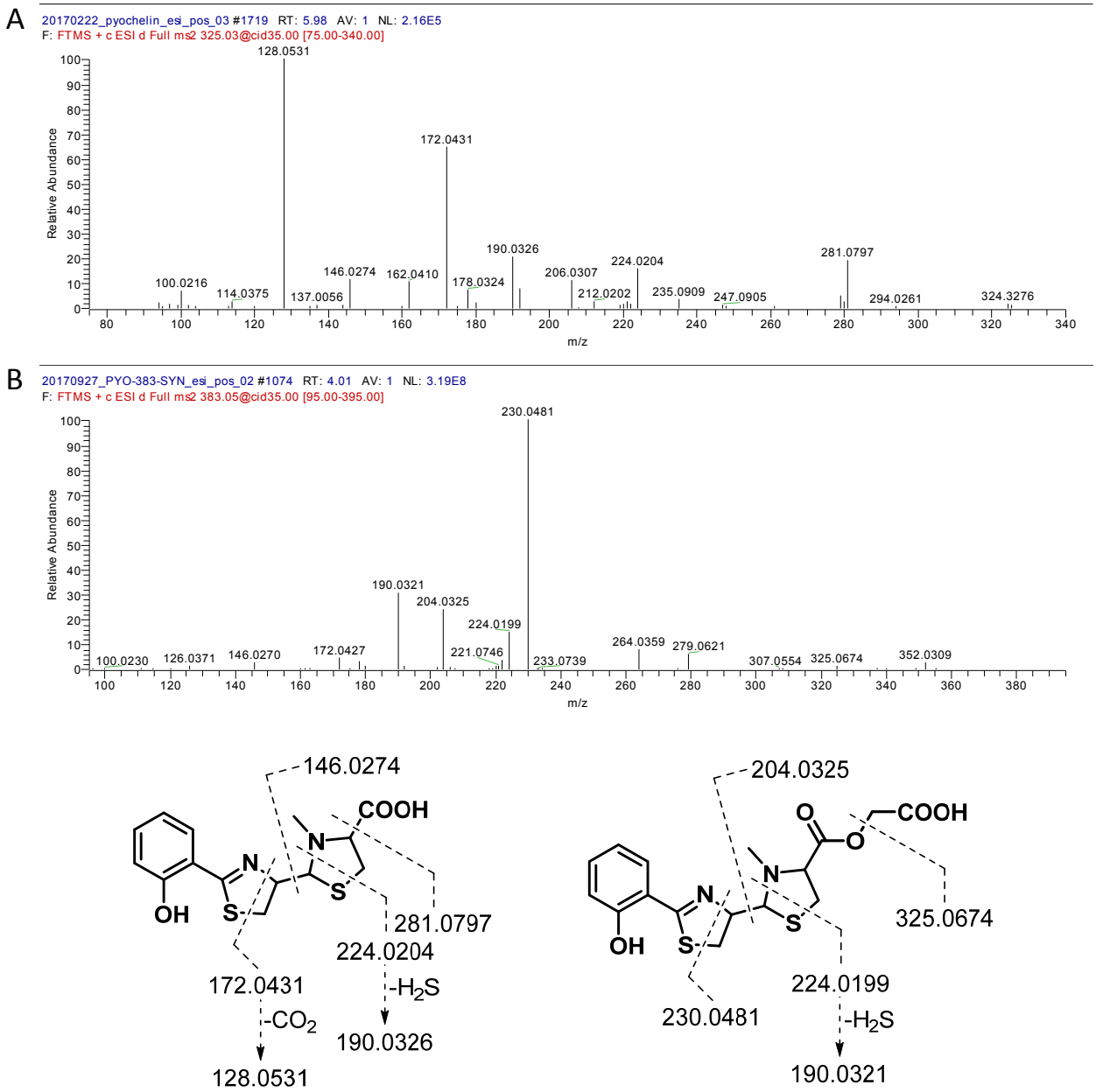


Fig. S7. HR-MS2 spectra and fragments of pyochelin (A) and pyochelin-GA (B).

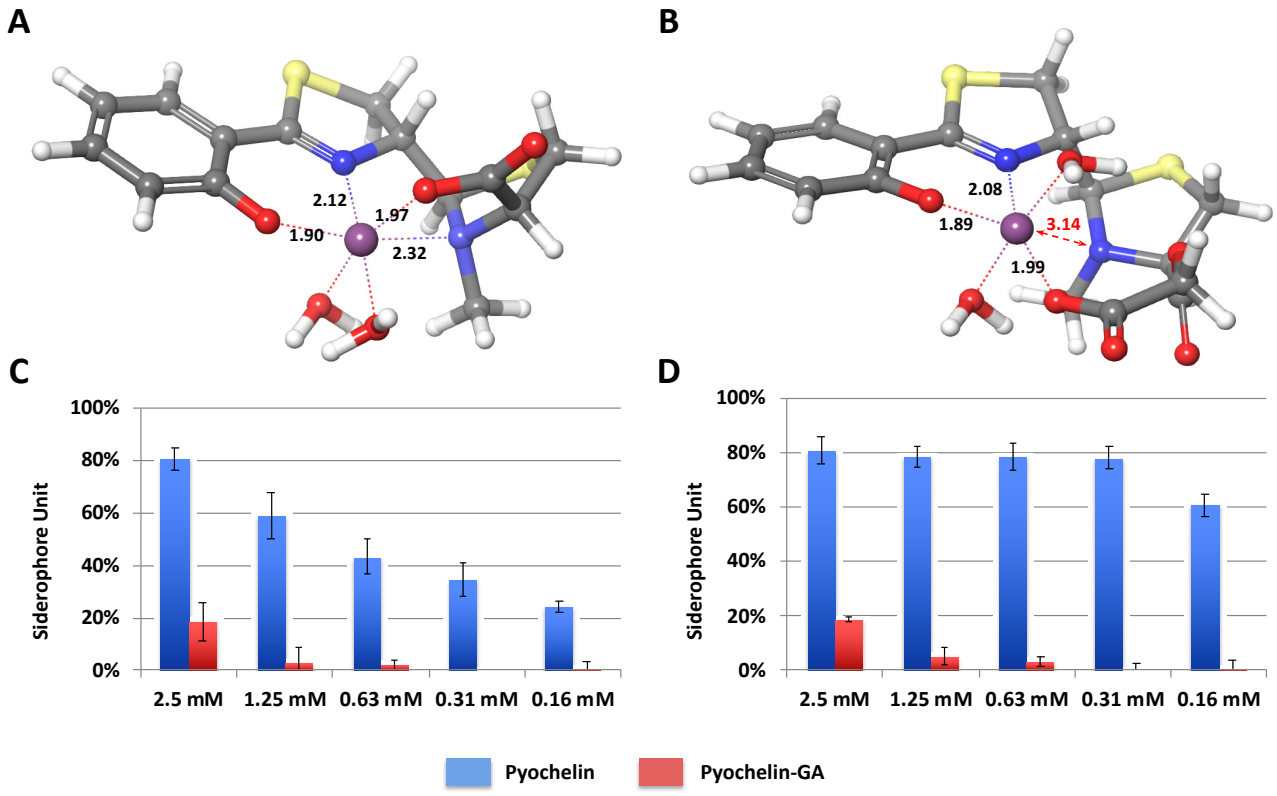
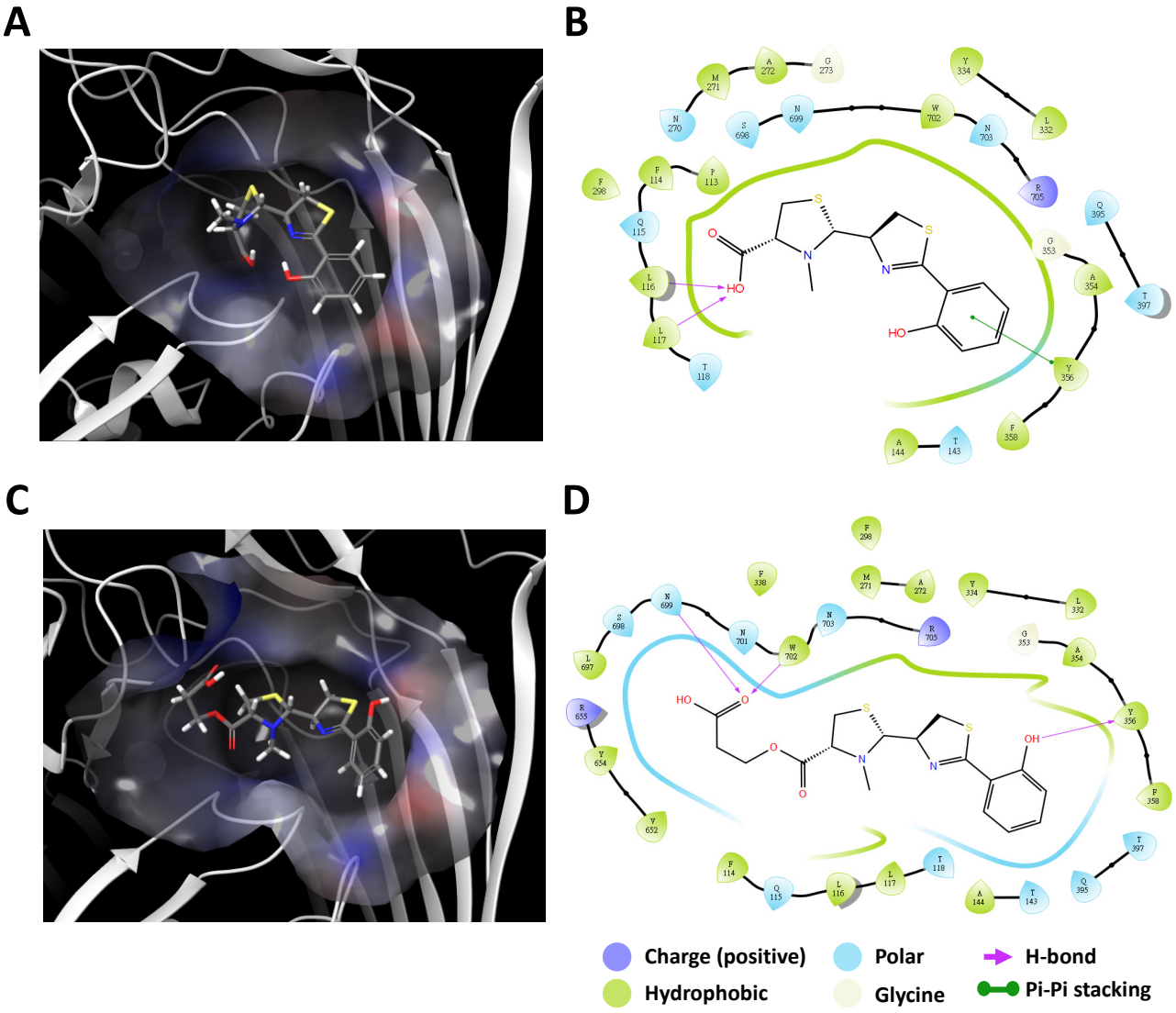


Fig. S8. The mean metal-ligand bond distances (in Å) of (A) Fe^{3+}-pyochelin complex and (B) Fe^{3+}-Pyochelin-GA complex evaluated using *In silico* B3LYP/6-31+G(3d,p). Siderophore unit (%) of pyochelin (blue) and pyochelin-GA (red) measured by using Chrome Azurol S assay at (C) 3 and (D) 24 hours. The siderophore unit was calculated according to the following formula: (Ar-As)/Ar × 100% (Ar = absorbance of the reference and As = absorbance of the tested sample).



E

Name	FptA-Pyochelin	FptA-Pyochelin-GA
Docking score (Glide)	-6.801	-4.348
Glide energy (kcal/mol; Glide)	-51.180	-40.369
MMGBSA dG Bind (kcal/mol; Prime)	-60.24	-42.64

Fig. S9. Outline of molecular docking analysis and 2D ligand interaction diagrams of pyochelin and pyochelin-GA with Fe(III)-pyochelin receptor (FptA) from *Pseudomonas aeruginosa*. (A) Pyochelin docking pose and (B) 2D ligand interaction diagrams within 6 Å in the binding site of FptA (1XKW); (C) Pyochelin-GA docking pose and (D) 2D ligand interaction diagrams within 6 Å in the binding site of FptA (1XKW); (E) Scoring and binding energy of Glide and MMGBSA.

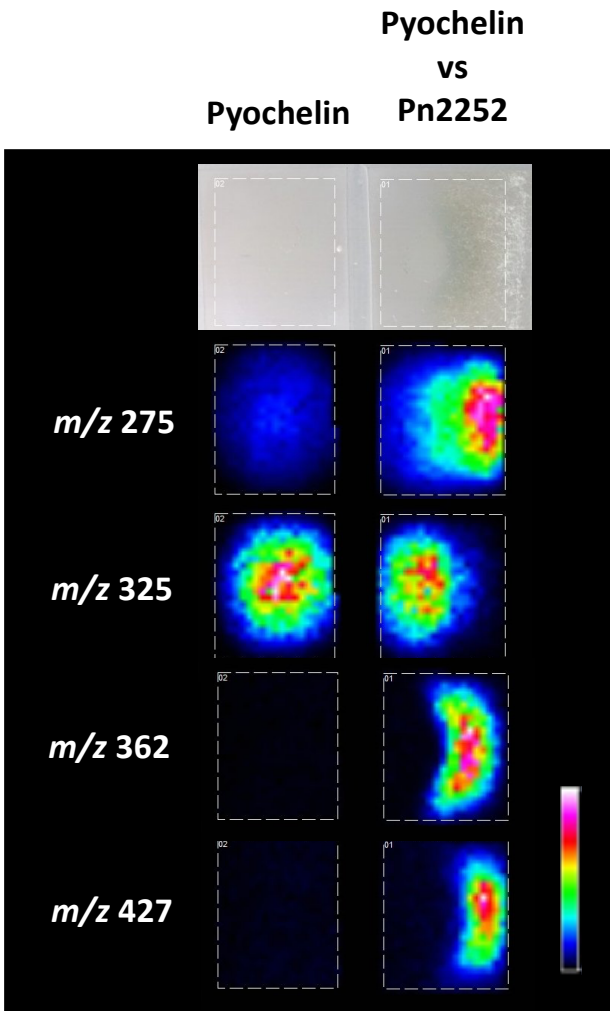


Fig. S10. The induced metabolites (*m/z* 275, *m/z* 362, *m/z* 427) of *P. noxius* 2252 (Pn2252) treated with 0.5 mg of pyochelin at 30 ˚C for 8 hours.

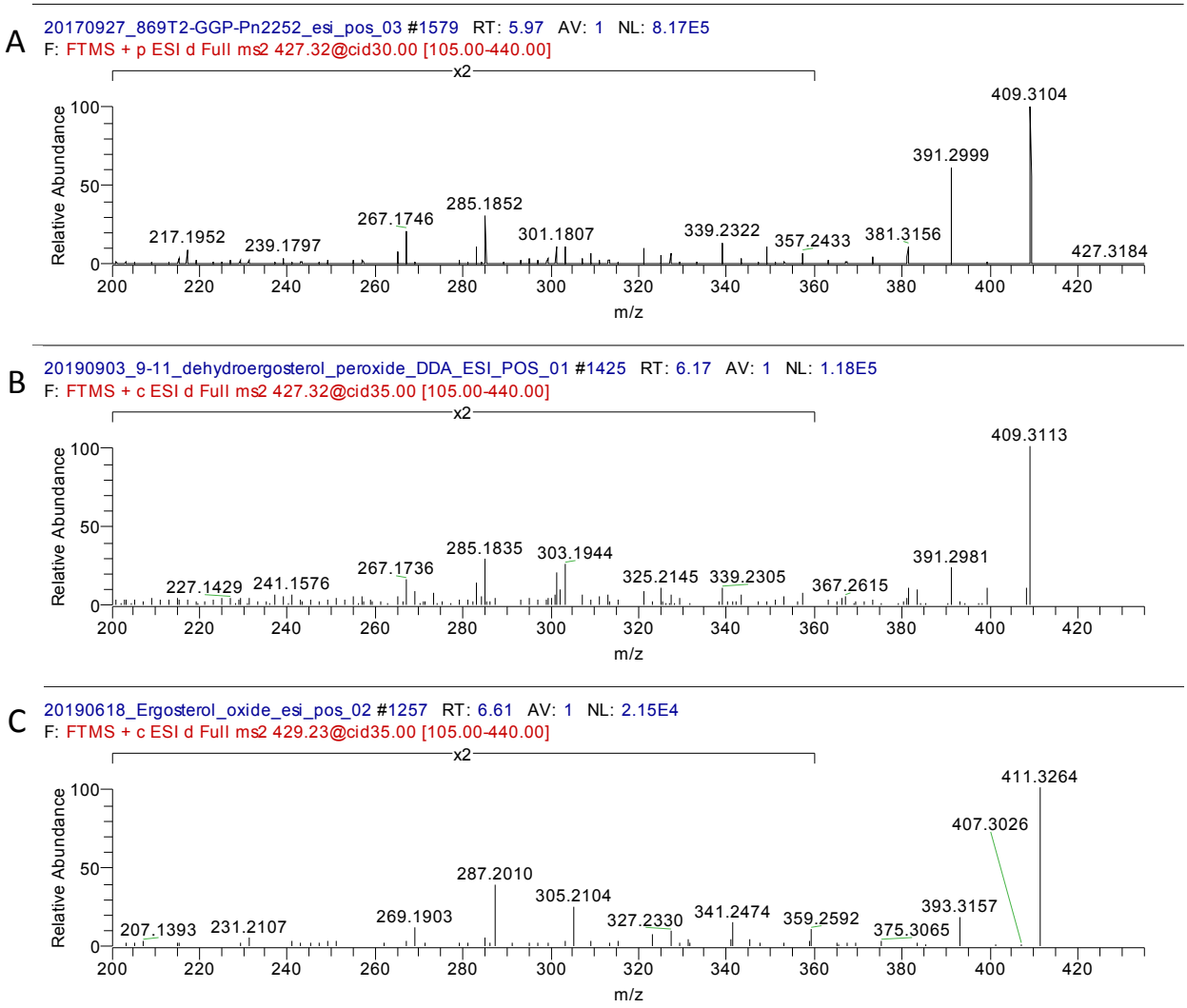


Fig. S11. HR-MS2 spectra of *m/z* 427 from dual-cultured samples (A) and 9,11-dehydroergosterol peroxide (B) and ergosterol peroxide (C). HRMS $[M+H]^+$: 427.3196 (calculated for $C_{28}H_{43}O_3$, 427.3212).

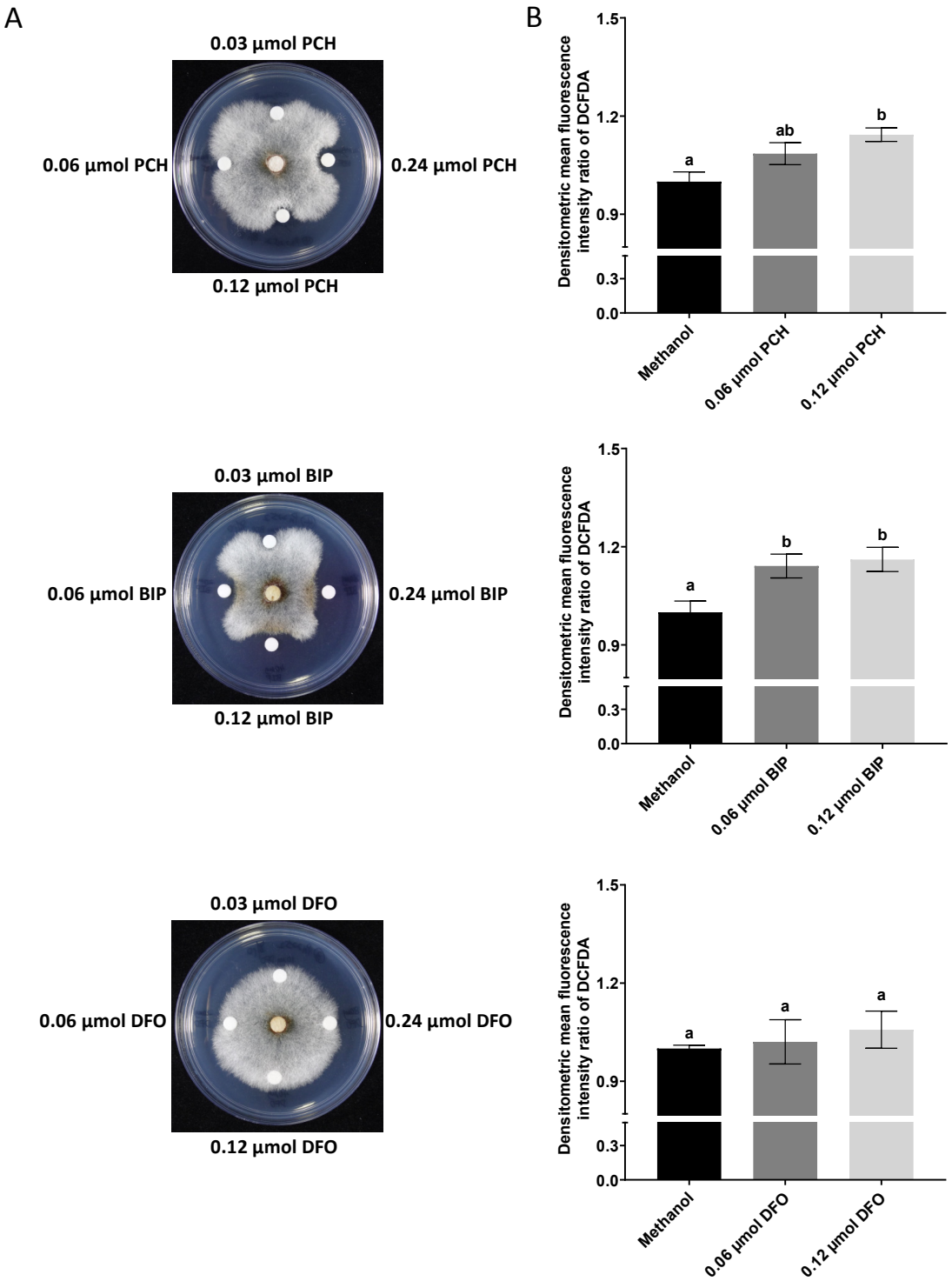


Fig. S12. Mycelia growth (A, 24 hours) and ROS generation (B, 4 hours) of *P. noxius* 2252 treated with pyochelin (PCH), 2,2′-bipyridyl (BIP), and deferoxamine (DFO) at 30 ˚C. DFO did not show antifungal activity and induce ROS production in *P. noxius* 2252. Therefore, we used DFO to prepare the iron-deficiency condition (Fig. 2). Statistical analyses were performed using GraphPad Prism 8 for Windows. One-way analysis of variance (ANOVA) followed by the Newman-Keuls test was used to determine statistical differences ($p < 0.05$) throughout the study. Different letters above each bar refer to the significant difference of multiple comparisons.

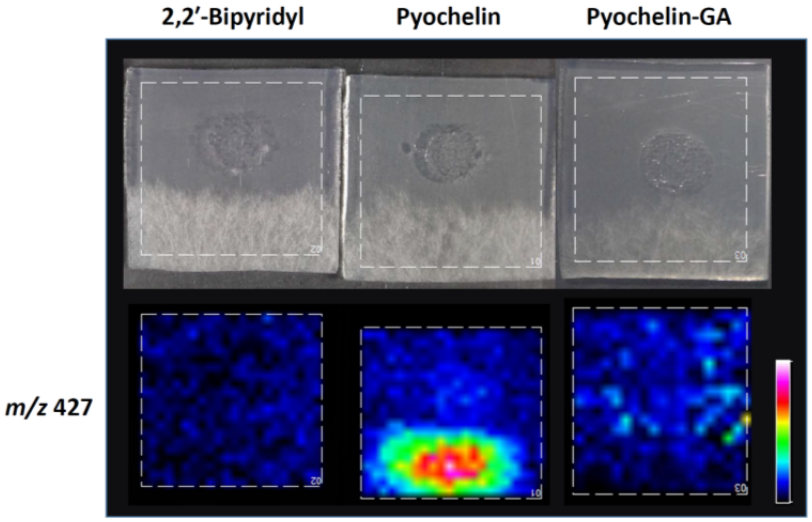


Fig. S13. MALDI-TOF IMS analysis of *P. noxius* 2252 treated with 0.12 µmol of 2,2′-bipyridyl, pyochelin, and pyochelin-GA at 30 ˚C for 6 hours, respectively.

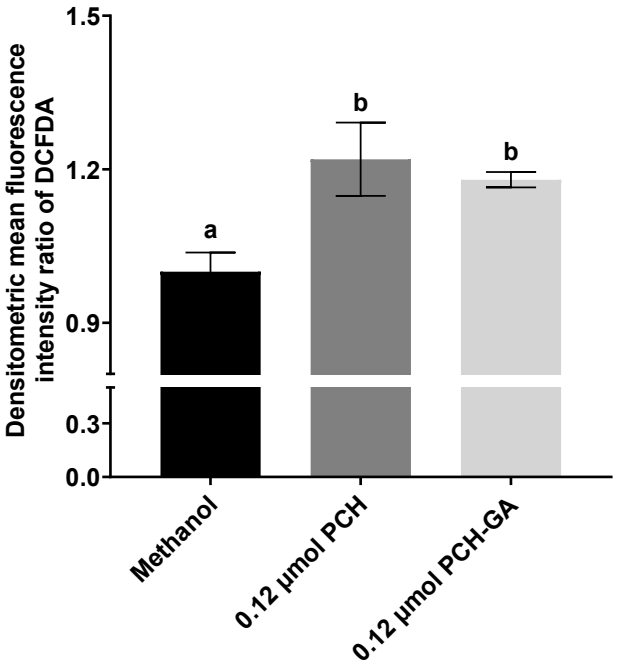


Fig. S14. Reactive oxygen species (ROS) production in *P. noxius* 2252 treated with pyochelin (PCH) and pyochelin-GA (PCH-GA) at 30 ˚C for 4 hours, respectively. Statistical analyses were performed using GraphPad Prism 8 for Windows. One-way analysis of variance (ANOVA) followed by the Newman-Keuls test was used to determine statistical differences ($p < 0.05$) throughout the study. Different letters refer to the significant difference within each bar.

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