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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Deltavision SoftWorx software 5.5.1 was used for collection of microscopy images (Figure 4, Supplementary Figure 9).
Data analysis	The custom R scripts developed for processing RB-TnSeq data described in this manuscript are available at https://github.com/DuttonLab/RB-TnSeq-Microbial-interactions along with usage instructions. The perl scripts used for initial processing of RB-TnSeq data published in Wetmore et al. 2015 are available at https://bitbucket.org/berkeleylab/feba/src/master/. Geneious version R9 9.1.8 was used for ribosomal subunit sequence alignment and the MrBayes plugin was used for creation of the phylogenetic tree in Figure 1; FigTree v1.4.4 was used for visualization. Global Natural Products Social Molecular Networking (GNPS), including the Dereplicator+ tool, was used to analyze mass spectrometry data and is available at https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp. XCMS Online version 2.7.2 (https://xcmsonline.scripps.edu/landing_page.php?pgcontent=mainPage) was used to make the cloud plot in Figure 6d. ClusterProfiler (R package version 3.12.0) was used for GO ontology functional enrichment analysis of bacterial gene sets. For P. psychrophila, a custom annotation database was created for use in ClusterProfiler using AnnotationForge (R package version 1.26.0). Cytoscape v. 3.5.1 was used to create the networks seen in Figures 3 and 5. R package ggfortify 0.4.7 was used to make PCA plots. R package UpSetR v. 1.4.0 was used to make UpSet plots. COG category mapping was done with eggNOG-mapper v2. Microscopy images in Figure 4 were deconvoluted using Deltavision SoftWorx 5.5.1 software, analyzed using Fiji ImageJ 1.52h, and assembled in Adobe Photoshop CC 2018. The following tools were used for RNA-Seq analysis: Geneious version R9 9.1.8, Rsamtools (R package version 2.0.3), GenomeInfoDb (R package version 1.20.0), GenomicFeatures (R package version 1.36.4), GenomicAlignments (R package version 1.20.1), GenomicRanges (R package version 1.36.1), DESeq2 (R package version 1.20.1), and KOBAS 3.0. Plots in Figures 2, 3, 5, and 6, Supplementary Figures 1, 2, and Extended Data 3, 5, 8, and 10 were made with R package ggplot2 3.2.1. AntiSMASH 5.0 was used for secondary metabolite biosynthesis cluster prediction. For Oxford Nanopore sequencing data, data was basecalled using guppy 3.3.0, assembled with canu 1.8, and polished with racon 1.4.3 and pilon 1.23.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data that support the findings of this study (RB-TnSeq, RNA-Seq) have been deposited in the NCBI SRA database with SRA accession codes SRR11514793-SRR11514872 and BioProject PRJNA624168. Mass spectrometry data is available in the MassIVE database under accession numbers MSV000085070 and MSV000085054. The GNPS molecular network is available at https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=464b331ef9d54de9957d23b4f9b9db14. The E. coli annotation database used for gene ontology functional enrichment is available at http://bioconductor.org/packages/release/data/annotation/html/org.EcK12.eg.db.html. The Whole Genome Shotgun project for Penicillium sp. str. #12 including reads, genome assembly, and annotation has been deposited at DDBJ/ENA/GenBank under the accession JAASRZ000000000 in BioProject PRJNA612335 (BioSample SAMN14369290 and SRA SRR11536435). In addition to these sources, data used to create figures 2,3,5, and 6 is available in the Supplementary Tables provided with the paper.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study uses clonal bacterial strains that are highly reproducible. We used at least 3 biological replicates for all experiments as it is standard practice for most microbiological assays. No sample size calculation was performed to determine sample size.
Data exclusions	No data were excluded from the analyses.
Replication	All competitive growth experiments, RB-TnSeq assays, RNA-Seq assays, CAS assays, biotin quantification, and mass spectrometry data collections were performed at least in triplicate from distinct samples. All attempts at replication were successful. For RB-TnSeq assays, the mutant library has a median of 16 (E. coli) or 18 (P. psychrophila) insertion mutants per gene, which can also be treated as replicates within each biological sample.
Randomization	Randomization is not relevant to and was not performed in this study. No group allocation occurred.
Blinding	Blinding is not relevant to and was not performed in this study. No group allocation occurred.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging