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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Agilent OpenLAB CDS Chemstation C.01.04 Agilent MassHunter workstation Data Acquisition BioTek Synergy/H1 microplate reader Gen5 2.08 CyBio Composer-CyBi-Well Studio Bruker_Biospin TopSpin V3.2.5 Thermo Tune Plus 2.7.0.1103 SP1 LDS LAESI Desktop Software 2.5.0.7
Data analysis	AntiBase 2013 Agilent OpenLAB CDS Data Analysis A.01.01 Agilent MassHunter Qualitative Analysis B.06.00 Microsoft Office Professional 2016 MestreLab Research MestReNova 12.0.1 PerKinElmer ChemDraw Professional 17.0 GraphPad Prism 5 Adobe Illustrator CC 2018 NCBI (www.ncbi.nlm.nih.gov) JGI IMG (img.jgi.doe.gov/cgi-bin/w/main.cgi) Unicycler GPL 3.0

SPAdes 3.11.1
AntiSMASH 4.0 (antismash.secondarymetabolites.org)
FramePlot 4.0beta (http://nocardia.nih.go.jp/fp4/)
RAST Server 2.0 (http://rast.nmpdr.org/)
NRPSpredictor2 (http://nrps.informatik.uni-tuebingen.de)
PKS/NRPS Analysis (http://nrps.igs.umaryland.edu/)
Protea Plot 2.0.1.3
Gubbs Mass Spec Utilities 8.4.32
MATLAB R2015b
CYLIB 2.0
CYANA 2.1
Pymol 2.2.0

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 upon request. 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

The data supporting the findings of this study are available within the paper and the supplementary material. NMR data used to characterize the cryptic metabolites are available from the corresponding author upon reasonable request. The DNA sequence of the ker gene cluster from A. keratiniphila has been submitted to GenBank (accession number MH428036). The LAESI-IMS data for S. canus and A. keratiniphila, including the raw data for Figs. 3a and 4a as well as the source code used to generate the 3D plots, have been submitted to GNPS (accession number MassIVE MSV000082658).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	High-throughput elicitor screening with each of the three bacterial strains followed by imaging mass spectrometry was carried out in a single replicate, as is typical for high-throughput screens. To ensure the hits observed in the high-throughput screen were genuine, they were validated in 96-well plate and flask cultures in 3 independent biological replicates, yielding excellent reproducibility. Typical examples of the validation results by HPLC-MS are shown in Figs. 2c, 3c, and 4c.
Data exclusions	No data were excluded from any analyses.
Replication	All experimental results could be reliably replicated.
Randomization	No experimental groups were used in this study, and randomization was therefore not relevant.
Blinding	Group allocation was not relevant to the experiments in this study.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

Pseudomonas protegens Pf-5 ATCC BAA-477
Amycolatopsis keratiniphila subsp. keratiniphila NRRL B-24117
Streptomyces canus NRRL B-3980
These bacterial strains are available from the American Type Culture Collection (ATCC) and/or the Northern Regional Research Laboratory (NRRL) culture collection.