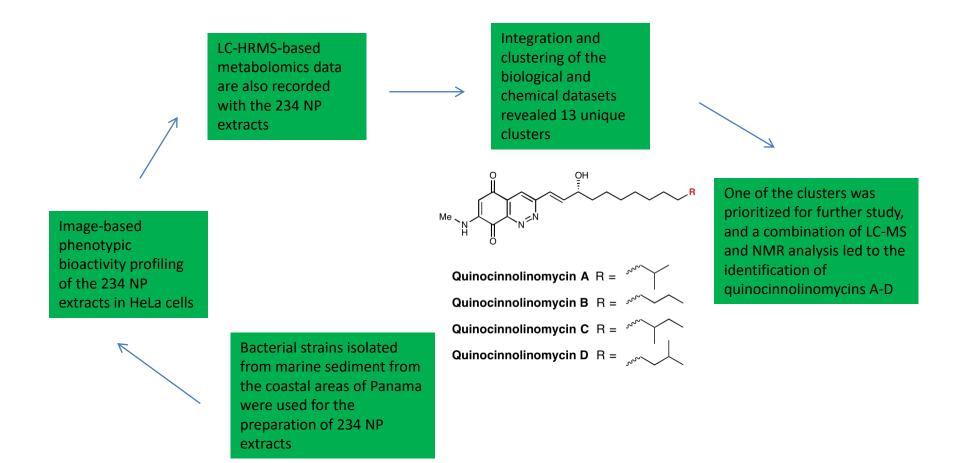
Figure 2A



Kurita, K. L., Glassey, E. & Linington, R. G. Integration of high-content screening and untargeted metabolomics for comprehensive functional annotation of natural product libraries. Proc. Natl. Acad. Sci. U. S. A. 112, 11999–2004 (2015).

Figure 2B

Transformation into heterologous expression host (*A. nidulans*) of the 56 FACs and preparation of NP extracts

Selected 56 FACs predicted to contain uncharacterized BGCs (i.e., BGCs with no known product or wellcharacterized homolog)

> Generation of 156 FACs containing unique BGCs from three species from the *Aspergillus* genus

15 new metabolites and their BGCs are characterized through combination of gene deletions within the BGCs and additional LC-MS and NMR analysis

Analysis of the metabolomics data with FAC-Score algorithm

which filters out signals present

in host extracts or in more than

one FAC strain.

The NP extracts are

HRMS

subjected to untargeted LC-

Clevenger, K. D. et al. A scalable platform to identify fungal secondary metabolites and their gene clusters. Nat. Chem. Biol. 13, 895–901 (2017).

Figure 3A

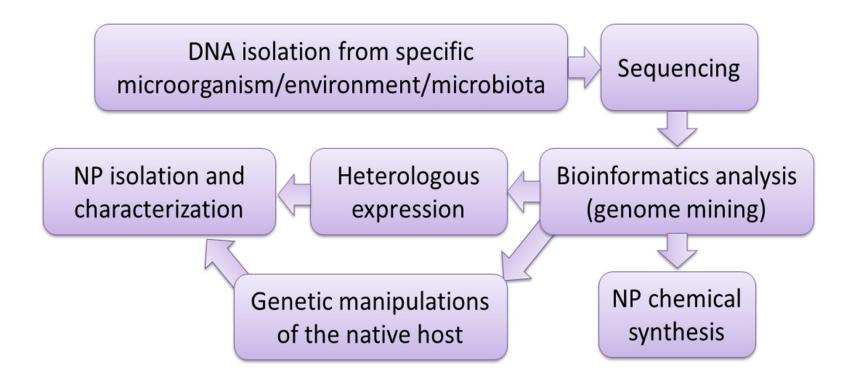
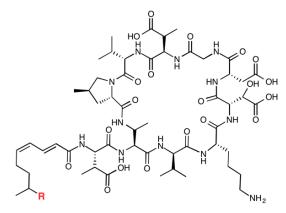
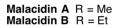


Figure 3B

Analysed sequences for BGCs that code for lipopeptides with calcium-binding motifs and clustered to create a phylogenetic tree Selected a soil sample rich in BGCs from a tree branch not associated with the BGCs for known calciumbinding antibiotics and cloned DNA in a cosmid library

Sequenced metagenomes of 2,000 soil samples





Malacidins isolated from cultures and their structures elucidated using a combination of mass spectrometry and NMR data, supported by bioinformatic analysis of the BGC Identified a predicted BGC and transferred into *Streptomyces albus* using transformationassociated recombination

Extracts from cultures of *S. albus* harbouring the BGC found to show antibacterial activity against *Staphylococcus aureus*

Hover, B. M. et al. Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. Nat. Microbiol. 3, 415–422 (2018).

Chemical synthesis of 25 "synthetic-bioinformatic" NP-like compounds that are predicted to be encoded of the analyzed gene clusters _____

Testing for antibacterial activity of the 25 "synthetic-bioinformatic" NPlike compounds against a panel of common human commensal and pathogenic bacteria

Humimycins are identified as new antibiotics active against methicillin-resistant Staphylococcus aureus

Identification of 57 unique nonribosomal peptide synthetase (NRPS) gene clusters



Genomic sequence data from human microbiome are bioinformatically queried for gene clusters predicted to encode large (≥5 residues) nonribosomal peptides

Chu, J. et al. Discovery of MRSA active antibiotics using primary sequence from the human microbiome. Nat. Chem. Biol. 12, 1004–1006 (2016).

Figure 4A

An extract from a new bacterial species, *Eleftheria terrae*, demonstrated good antimicrobial activity against *S. aureus* and was chosen for further study

Compound isolation from the *E. terrae* extract, and structure elucidation by NMR and advanced Marfey's analysis yielded teixobactin, a new antibiotic with activity against Gram-positive bacteria

Extracts from 10,000 iChipisolates were screened for antimicrobial activity against *Staphylococcus aureus*

Teixobactin

iChip device with diluted soil samples is incubated in the soil with the goal to simultaneously isolate and grow uncultured bacteria

Ling, L. L. et al. A new antibiotic kills pathogens without detectable resistance. Nature 517, 455–459 (2015).

Figure 4B

Antigens representative for the selected extracellular protein domains are designed, synthesized, and injected in rabbits for antibody production

IgG is purified from the rabbit serum and is fluorescently labelled

> Oral microbiota samples are subjected to staining with the fluorescently labeled antibodies followed by flow cytometry and cell sorting in order to isolate the targeted bacterial species

Selection of microbial species from human oral microbiome for targeted isolation based on genomic sequence data This reverse genomics workflow was validated by the isolation and cultivation of three species of TM7/Saccharibacteria along with their interacting Actinobacteria hosts, as well as SR1 bacteria that are members of a candidate phylum with no previously cultured representatives

Cross, K. L. et al. Targeted isolation and cultivation of uncultivated bacteria by reverse genomics. Nat. Biotechnol. (2019). doi:10.1038/s41587-019-0260-6

Bioinformatics analysis to identify membrane proteins with extracellular domains that could serve as antigens for antibody development