The planctomycete *Stieleria maiorica* Mal15<sup>T</sup> employs stieleriacines to alter the species composition in marine biofilms

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Bacterial strains of the phylum Planctomycetes occur ubiquitously, but are often found on surfaces of aquatic phototrophs, e.g. alga. Despite slower growth, planctomycetes are not outcompeted by faster-growing bacteria in biofilms on such surfaces; however, strategies allowing them to compensate for slower growth have not yet been investigated. Here, we identified stieleriacines, a class of N-acylated tyrosines produced by the novel planctomycete *Stieleria maiorica* Mal15<sup>T</sup>, and analysed their effects on growth of the producing strain and bacterial species likely co-occurring with strain Mal15<sup>T</sup>. Stieleriacines reduced the lag phase of Mal15<sup>T</sup> and either stimulated or inhibited biofilm formation of two bacterial competitors, indicating that Mal15<sup>T</sup> employs stieleriacines to specifically alter microbial biofilm composition. The genetic organisation of the putative stieleriacine biosynthetic cluster in strain Mal15<sup>T</sup> points towards a functional link of stieleriacine biosynthesis to exopolysaccharide-associated protein sorting and biofilm formation.

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From a heterotrophic bacterial perspective, the vastness of the sea represents a hostile oligotrophic ‘desert’. In contrast, surfaces of marine macroscopic phototrophs are nutrient-rich ‘oases’, densely packed with all sorts of alluring organic compounds, which can serve as nutrient sources. In aquatic environments, such biotic surfaces represent desirable ecological niches and are thus rapidly occupied by bacterial biofilms1,2. Members of the ‘Roseobacter group’ are particularly successful in such habitats3. For example, *Phaeobacter inhibens* employs *N*-acyl homoserine lactones (AHLs) as quorum sensing signals to initiate biofilm formation4. In *P. inhibens*, AHLs trigger the expression of the gene encoding AHL synthase along with other genes that promote biofilm formation. This positive feedback loop is often associated with the production of bioactive small molecules, e.g., the antibiotic tropidethiatic acid (TDA) by *P. inhibens*5,6.

While *P. inhibens* and other proteobacteria are well known to dominate biotic surfaces, it was found that members of the phylum Planctomycetes can sometimes also be the dominating taxon7. Species belonging to the family Pirellulaceae, including other microorganisms9. Large planctomycetal genomes of up to 12.4 Mb and high numbers of predicted clusters involved in small molecule production are in line with the assumed portfolio of bioactive compounds with potential regulatory activities, in addition to two component systems and extracytoplasmic function sigma factors10. Although planctomycetes grow rather slowly compared to competing microorganisms occupying the same ecological niche, they are not outcompeted by their natural competitors. On the contrary, planctomycetes can even account for up to 70% of the bacterial community in certain habitats7. The deficit in growth rate is suggested to be compensated by the production of small molecules with antimicrobial properties, while the chemical nature of such molecules remains elusive11. The current knowledge gap mainly results from the insufficient number of planctomycetes available as axenic cultures7. Recently, we developed an isolation pipeline to obtain novel planctomycetal strains in axenic culture. Basis for the isolation of 79 novel strains was an optimised formulation of cultivation media, supplemented with a blend of carefully titrated antibiotics and fungicides10. In *Mal15*7, one of the strains isolated by this strategy, we detected production of novel compounds belonging to the class of *N*-acylated tyrosine derivatives, which might have regulatory activities in this strain. In order to validate our working hypothesis, we performed assays with purified stieleriacine for studying its role on intraspecies and interspecies communication. Additionally, we inferred a putative biosynthetic pathway based on the genome sequence of strain *Mal15* obtained during a detailed characterisation of the novel strain.

### Results

**Description of Stieleria maiorica sp. nov. Stieleria maiorica** (ma."ori.ca") N.L. fem. adj. *maiorica*, pertaining to the island Mallorca, Spain, on which the type strain was isolated). In addition to the features described above the species exhibits the following properties. Colonies are pink-coloured on solid medium. Cells are 1.9 ± 0.2 x 1.4 ± 0.2 μm in size. Motile daughter cells originate through budding from sessile mother cells. Gram staining delivers no clear results. The oxidase assay was negative while the catalase assay was positive. The organism can degrade a wide range of carbon sources. In particular, strong signals were observed for *N*-acyl-d-galactosamine, *N*-acyl-d-glucosamine, l-arabinose, d-cellobiose, l-fucose, d-fructose, d-galactose, gentiobiose, d-galactosyl-d-glucosyl acid, glucuronamide, d-glucuronic acid, d-lactose, lactulose, d-mannose, d-melibiose, β-methyl-d-glucoside, d-rafinose, l-rhamnose, sucrose, d-trehalose, turanose and d-psicose, while for the carbon sources acetic acid, dextrin, d-galactonic acid lactone, d-glucose-6-phosphate, α-ketoglutaric acid, maltose, d-mannitol only weak signals were detected. The enzyme repertoire includes alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase. Growth of the type strain occurs between pH 5.5 and 9.0 with an optimum at pH 7.5. The optimal growth temperature of the type strain was determined to be 35 °C, but cells could grow in a range from 11 °C up to at least 37 °C. The complete chromosome of the type strain comprises 9,894,293 bp, with a G+C content of 59.3%. The type strain is *Mal15* (DSM 100215T = LMG 29790T, synonym Malle15), which was isolated from seawater sediment on Mallorca island, Spain.

**Identification of stieleriacines in cultures of strain Mal15T.** Given our working hypothesis that planctomycetes are a promising source for novel bioactive small molecules, we continuously extend the current collection of axenic cultures from this phylum10. Here, we analysed the novel biofilm-forming strain *Mal15* (DSM 100215T = LMG 29790T) isolated from sediments on Mallorca island in the Mediterranean Sea. Our phylogenetic analysis suggests that strain *Mal15* belongs to a novel genus and species in the family Pirellulaceae. Hence, we propose the name *Stieleria maiorica* gen. nov., sp. nov. for the novel isolate. A detailed characterisation of the novel strain is presented in the Supplementary Information.

During metabolite analysis in culture supernatants of strain *Mal15*, we found that it produces a distinct class of small molecules belonging to the group of long-chain *N*-acyl tyrosine derivatives, which we named stieleriacines (in accordance with the proposed genus name *Stieleria*). Stieleriacines are composed of a lauric acid or *trans-2*-dodecenic acid moiety as fatty acid residue, which is ligated to a dehydrotyrosine derivative additionally C-methylated in *meta*-position of its aromatic ring (Fig. 1). Stieleriacines were found to be produced during laboratory-scale shaking flask cultivations of axenic *Mal15* cultures, indicating that their production does not require presence of other microorganisms as an external stimulus.

**Analysis of in vitro and in vivo effects of stieleriacine A1.** In previous studies, it has been shown that *N*-acyl amino acids can display antimicrobial activities against various bacteria12–17,
while N-acylated tyrosine derivatives were shown to inhibit the enzyme tyrosinase. Employing in vivo and in vitro assays with purified stieleriacine A1, the major stieleriacine produced by strain Mal15T, we could show that these compounds exhibit only moderate antimicrobial activity against Gram-positive bacteria (Supplementary Table 1) and negligible inhibition of tyrosinase, indicating that neither of the two effects reflects the major function of stieleriacines. To get first hints on the natural function of stieleriacine A1, we thus applied time-lapse microscopy coupled to microfluidics as experimental strategy to allow observation of individual cells over time spans of several hours to days. Such unusual approaches were necessary since most of the canonical biofilm formation assays did not work for planktomycetes. Mal15T cells were treated with 1.34 µM stieleriacine A1, a ‘physiological’ concentration found to be produced in the mid-exponential phase during laboratory-scale cultivation of the strain (Fig. 2). The natural production of stieleriacines by strain Mal15T can be neglected in this experimental setup as a constant medium flow ensures that compounds secreted by the strain are immediately flushed away. We found that presence of stieleriacine A1 significantly reduced the duration of the lag phase (5.2 h) compared to untreated Mal15T cells (6.3 h; p = 0.0058) (Fig. 3, Supplementary Table 2, Supplementary Movie 1 and 2). This observation points towards a bioactivity to the advantage of its producer, however, we did not exclude any additional inhibitory effect on competing microbes at this stage.

The artifical laboratory cultivation conditions and the tested concentration of stieleriacine A1 probably only insufficiently reflect natural conditions in seawater, in which densities of planktonic Mal15T cells are much lower. From an ecological perspective, stieleriacine production by individual swarming cells or smaller aggregates appears irrational as the secreted molecules are immediately diluted to inactive concentrations. We hypothesised that sufficient stieleriacine concentrations can only be reached by larger numbers of cells inhabiting a local environment, such as a biofilm with a rather gel-like texture and that stieleriacines might thus play a more important role in such micro-environments. Following this argumentation, we were curious to test for a potential effect of stieleriacine A1 on biofilm formation capabilities of natural competitors of strain Mal15T. We chose two members of the ‘Roseobacter group’, P. inhibens DSM 17395 and Sulfitobacter dubius DSM 16472T, which were shown to co-occur with species of the family Pirellulaceae.

The biofilm assays were performed with isolated stieleriacine A1, in absence of Mal15T cells to exclude any additional effects, which might e.g. be caused by other natural compounds produced by strain Mal15T. Unexpectedly, application of stieleriacine A1 led to an increased biofilm formation of P. inhibens (+35%, p < 0.001), but reduced biofilm formation of S. dubius (-15%, p < 0.001) (Fig. 4a). This effect was observed when 134 µM stieleriacine A1 was used, a 100-fold higher concentration than in the microfluidics experiment performed with strain Mal15T. The biofilm assay required empirical optimisation of the stieleriacine A1 concentration; since we were aware that concentrations in liquid
cultures might not properly reflect local concentrations in biofilms, in which cells are in very close proximity and local metabolite concentrations are thus considerably higher. We decided to follow the empirical optimisation approach of the stieleriacine A₁ stimulation to reconstruct the natural conditions in the biofilm as good as possible. Natural local concentrations of stieleriacines in biofilms are difficult to assess and largely dependent on size and microbial community composition of the biofilm. The observed stimulating effect of stieleriacine A₁ on *P. inhibens* was surprising and was thus analysed further. To this end, we tested for a potential interaction of stieleriacine A₁ with the AHL-dependent quorum sensing system in *P. inhibens*, in particular the AHL-responsive transcriptional regulator protein LuxR. Mutant analysis with a luxR-deficient *P. inhibens* strain (Supplementary Fig. 1) demonstrated that the positive effect on biofilm formation is largely independent from LuxR, indicating that stieleriacine A₁ does not directly interfere with the quorum sensing system of *P. inhibens* (Fig. 4b). But, why should strain Mal15ᵀ produce a molecule that stimulates biofilm formation of its natural competitor *P. inhibens*, while reducing the fitness of its other competitor *S. dubius*? The reason possibly relates to production of TDA (22). Promoting the biofilm formation of *P. inhibens* would in return lead to increased production of AHLs and TDA (23, 24). This benefit strain Mal15ᵀ in two ways: (i) Mal15ᵀ turned out to be resistant against TDA while other competitors are not. (ii) TDA production reduces the growth speed of *P. inhibens* approximately by 41% due to the increased metabolic burden (25) and may ensure that strain Mal15ᵀ is not outcompeted. Thus, we suggest that strain Mal15ᵀ ‘invites’ *P. inhibens* via stieleriacine production to join the biofilm and to produce TDA in order to challenge other faster growing competitors. In contrast, biofilm formation of *S. dubius* is reduced by stieleriacine A₁ as this bacterium does not produce TDA and is in that regard useless for strain Mal15ᵀ. The hypothesis suggests that planctomycetes actively shape the biofilm community to gain advantages employing a novel type of natural products: stieleriacines. Our results thus point towards the capability of certain slow-growing bacteria to mediate colonisation of their surrounding by production of small molecules. The hypothesis fits to the observation that slow-growing planctomycetes can dominate biofilms on competitive marine surfaces (7).

**Genome analysis identifies a putative stieleriacine biosynthesis pathway.** Although microbial synthesis of N-acyl tyrosine molecules has been reported in several recent studies (18, 26, 27), the genetic basis for biosynthesis has not been elucidated in greater detail. The key enzyme for stieleriacine biosynthesis in strain Mal15ᵀ is likely an N-acyl amino acid synthase (NAS) (Fig. 5). We sequenced the 9.9 Mb genome of strain Mal15ᵀ and identified a cluster (locus tags Mal15_37240 to Mal15_37430, Fig. 6), which contains not only four putative NAS-encoding genes, but all genes required for a postulated stieleriacine biosynthesis pathway. The NAS-encoding genes (Mal15_37340, Mal15_37370, Mal15_37380 and Mal15_37430) were originally annotated as hypothetical proteins, but were identified as candidates by BLASTp and InterPro scan. The four enzymes might have different substrate spectra, which can explain the production of five slightly different stieleriacines in strain Mal15ᵀ (Fig. 1). However, it should not be excluded that the strain is capable of producing other N-acyl amino acids. The C-methylation in meta-position of the aromatic ring and an uncommon double bond in the αβ-position of the tyrosine moiety were identified as characteristic molecular features separating stieleriacines from other N-acyl tyrosines identified so far (18, 26). In the biosynthetic cluster, Mal15_37250 encodes a putative methyltransferase similar to the L-tyrosine C3-methyltransferase SfmM2 of *Streptomyces lavanu*. This enzyme catalyses an early step in saframycin biosynthesis (28) identical to the reaction required during synthesis of stieleriacines (Fig. 5). The gene encoded immediately downstream, Mal15_37560, codes for a putative tRNA threonylcarbamoyladenosine dehydratase. When only taking the putative protein annotation into consideration, involvement in stieleriacine biosynthesis appears rather unlikely. However, the substrate of the dehydratase (tRNA threonylcarbamoyladenosine) strongly resembles the amide bond obtained after the N-acylation reaction in the postulated stieleriacine pathway. The catalysed lactonization and dehydration reaction would give rise to a cyclic pathway intermediate, in which the remaining double bond can be introduced by a coupled oxidation and hydrolysis reaction.
induced by a keto-enol tautomeric rearrangement (Fig. 5). This reaction may be catalysed by the putative oxidoreductase encoded by Mal15_37310. Other proteins in the cluster code for a putative transcriptional regulator or putative transporters (Supplementary Table 3), which could have additional function for regulation of the pathway and product export, however, we had no clear indication for this based on published information. Mal15_37240, encoding a putative fatty acid desaturase, is likely involved in the introduction of the double bond in the fatty acid part of the molecule (Fig. 5), however it remains to be elucidated whether this reaction takes place before or after the N-acylation.

Discussion

In this study, we isolated and characterised Stieeleria maiorica Mal15T, which represents a novel species and genus within the family Pirellulaceae, order Pirellulales, class Planctomycetia, phylum Planctomycetes. The strain was shown to produce N-acylated tyrosine derivatives, which we designated stieleriacines and which belong to the class of N-acylated amino acids. In previous studies, compounds of this class showed antimicrobial activities against various bacteria12–17 and probably act as signalling molecules mediating intraspecies and interspecies communication4,15,29. N-acylated tyrosine derivatives isolated from the marine γ-proteobacterium Thalassotalea sp. PP2-459 (Alteromonadales) were also shown to inhibit the enzyme tyrosinase18. Recently, an N-acyl tyrosine congener harbouring an uncommon α-methyl group in the tyrosine moiety was identified in Alteromonas sp. RKMC-009 isolated from the surface of a marine sponge26, while its natural function remains to be elucidated. Stieleriacine A1 showed only moderate antimicrobial activity against Gram-positive bacteria and negligible tyrosinase inhibitory activity. At this early stage of research with the novel isolate, we can only speculate on potential quorum sensing activity of stieleriacines. Analysis of potential target genes of a respective regulator part of a putative quorum sensing system is challenging given that 42% (2897 out of 6920) proteins encoded in the genome of strain Mal15T are of unknown function. Experiments with the strain itself instead of isolated stieleriacine are particularly interesting, however, these require a Mal15T mutant incapable of stieleriacine biosynthesis as negative control. Such a mutant can also provide important information on the stieleriacine biosynthesis pathway, however, due to lacking experience in terms of the genetic accessibility of strain Mal15T, this question needs to be addressed in follow-up studies.

Although some N-acyl amino acid compounds have been identified, the current knowledge on the underlying biosynthetic pathways is limited. Evidence for the presence of N-acylated derivatives of aromatic or basic amino acids was mainly based on a multitude of environmental DNA libraries. Characterisation of biosynthetic pathways for N-acyl amino acids is an important task in the future given that such compounds find potential biotechnological applications as antimicrobial surfactants and precursors for biodegradable polyesters in pharmaceutical and biomedical applications30,31.

![Fig. 5 Proposed biosynthesis pathway of stieleriacines.](image)

**Fig. 5 Proposed biosynthesis pathway of stieleriacines.** The proposed stieleriacine biosynthesis pathway starting from L-tyrosine and lauryl-ACP is depicted. The locus tags of genes coding for candidate enzymes participating in the pathway are shown in boxes. ACP acyl carrier protein.

![Fig. 6 The putative stieleriacine biosynthetic gene cluster in strain Mal15T.](image)

**Fig. 6 The putative stieleriacine biosynthetic gene cluster in strain Mal15T.** Genes with a predicted role in the stieleriacine biosynthesis cluster are shown in different colours. For these genes the automated gene annotation and the postulated function are shown in the table. The complete list of automated gene annotations of the cluster can be found in Supplementary Table 3.
have the optimum antibacterial activity among the single chain surfactants tested\(^{35}\). In that regard, genes of strain Mal15\(^T\) are interesting target for expression in heterologous microbial hosts.

A report on screening of soil metagenomic libraries revealed that genes coding for NAS proteins are frequently found adjacent to genes involved in the PEP-CTERM/exosortase protein sorting system (an exopolysaccharide-associated protein targeting system), indicating a functional link between both pathways\(^{29}\). Indeed, the same situation is also found in the genome of strain Mal15\(^T\). A PEP-CTERM motif protein-encoding gene (Mal15_37300) was found within the proposed stieleriacine biosynthetic gene cluster (Fig. 6), and many exopolysaccharide biosynthesis-related and secretion-related genes are encoded in the region upstream (Mal15_36520 to Mal17_37230, Supplementary Table 3). The conserved genetic organisation supports the notion of a functional link between stieleriacine biosynthesis, exopolysaccharide assembly and biofilm formation, which requires additional attention in future studies.

In conclusion, the identified stieleriacines stimulated growth inhibition of Mal15\(^T\) cells and either promoted or inhibited biofilm formation of two different microorganisms, likely naturally competing with strain Mal15\(^T\) for biotic surfaces in marine environments. Although the two tested competitors might only be a small fraction of microorganisms dwelling in such environments, it allows first insights into strategies how slow-growing microorganisms compensate for lower growth rates and avoid being outcompeted. Stieleriacines are likely only one of several strategies how the survival of planctomycetes in aquatic environments is ensured and their high abundance on several marine biotic surfaces can be explained.

**Methods**

**Sample collection and preparation.** Seawater sediment samples from 30 cm depth were collected from Spain, Mallorca island, El Arenal, between Balneario 4 and 5 (39° 30’ 45.2” N, 2° 44’ 49.1” E) on September 23th, 2014. Samples were collected in sterile 50 mL polypropylene tubes, transferred to the laboratory, homogenised and processed.

**Culture and isolation conditions.** For maintenance of strain Mal15\(^T\) M1 medium with HEPES (H) as buffering agent and additionally supplemented with N-acetylglucosamine (NAG) and artificial seawater (ASW) (M1H NAG ASW medium) was used and prepared as described\(^{32}\). Solid medium was prepared with 12 g/L (w/v) agar, washed three times with double distilled H\(_2\)O and cooled to 55 °C prior to the addition of heat-sensitive solutions. For the initial strain isolation, solid M1H NAG ASW medium was supplemented with 100 μL of an ampicillin (50 mg/mL) and cycloheximide stock solution (20 mg/mL), dried for 30 min, inoculated with 100 μL homogenised sample material per plate and incubated at 28 °C and 80 rpm in 2 L baffled flasks until 2% (w/v) of purified adsorbent resin XAD-16 (Rohm and Haas) was added. Cultures were further incubated for three days. In total, XAD-16 from 32 mL culture was harvested and processed as previously described\(^{32}\). The combined crude extract was filtered with water/methanol (1:1, v:v) and using the CellASIC ONIX Micro 1200 series HPLC-UV system in combination with an ESI-TOF-MS (Maxis, Thermo Scientific) was used for bacteria. Tested species were inoculated to initial cell densities of 10\(^5\) colony-forming units per mL in the respective growth medium. MIC were assessed after 16 and 48 h of cultivation at 37 °C (bacteria) and 28 °C (yeasts and moulds), respectively.

**Phylogenetic analysis.** 16S rRNA gene sequence-based phylogeny was computed for strain Mal15\(^T\) (GenBank acc. no. MK554562), the type strains of all described stieleriacine producers (Supplementary Table 3). The conserved genetic organisation supports the notion of a functional link between stieleriacine biosynthesis, exopolysaccharide assembly and biofilm formation, which requires additional attention in future studies.

**Isolation and purification of stieleriacines from culture broth.** For getting sufficient amounts of Mal15\(^T\) biomass, nutrient content of the culture broth was increased (1.0 g/L peptone, 1.0 g/L yeast extract, 40 mL/L 2.5% (w/v) glucose solution). Six hundred micro litre Mal15\(^T\) cultures were incubated for three days at 28 °C and 80 rpm in 2 L baffled flasks until 2% (w/v) of purified adsorbent resin XAD-16 (Rohm and Haas) was added. Cultures were further incubated for three days. In total, XAD-16 from 32 mL culture was harvested and processed as previously described\(^{32}\). The combined crude extract was filtered with water/methanol (1:1, v:v) and using the CellASIC ONIX Micro 1200 series HPLC-UV system in combination with an ESI-TOF-MS (Maxis, Thermo Scientific) was used for bacteria. Tested species were inoculated to initial cell densities of 10\(^5\) colony-forming units per mL in the respective growth medium. MIC were assessed after 16 and 48 h of cultivation at 37 °C (bacteria) and 28 °C (yeasts and moulds), respectively.

**Antimicrobial activity assay.** Determination of minimum inhibitory concentration (MIC) was performed using serial dilution assays in 96-well microtiter plates. YMO medium (4 g/L yeast extract, 10 g/L malt extract, 4 g/L glucose, pH 7.2) was used for bacteria and filamentous fungi and YMG medium (4 g/L yeast extract, 10 g/L malt extract, 40 mL/L 2.5% (w/v) glucose and pH 7.2) for yeasts and moulds. After 175 MHz, 175 MHz, 15N 71 MHz) UV spectra were recorded using a Shimadzu UV-VIS spectrophotometer UV-2450. Optical rotation was determined using a PerkinElmer 241 polarimeter. Correlations are shown in Supplementary Fig. 8.

**Cytotoxicity assay.** In vitro cytotoxicity (IC\(_{50}\)) was investigated using mouse fibroblast cell line L929 and HeLa KB3.1 cells as previously described\(^{48}\). Briefly, cells were cultivated in EBM-2 supplemented with 10% foetal bovine serum under 10% CO\(_2\) at 37 °C. Forty micro litre of serial dilutions from an initial stock of 1 mg/mL was incubated in acetonitrile at concentration of 120 μL aliquots of a cell suspension (50,000 cells per mL) in 96-well microtiter plates. After 5 days of incubation, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. The absorbance was measured at 590 nm using an ELISA plate reader (Victor). The concentration, at which the growth of cells was inhibited to 50% of the control (IC\(_{50}\)), was obtained from the dose–response curves. Acetone served as negative control.

**Tyrosinase inhibition assay.** The tyrosinase inhibition assay was performed as previously described\(^{48}\) with the following modifications: 80 μL of compound,
dissolved in PBS (0.1 M, pH 6.8) with 10% methanol at various concentrations, were added to 80 µL of PBS. PBS with 10% methanol served as a negative control. Eighty microlitre of mushroom-tyrosinase (100 units per mL) and 40 µL of 132 µL acetone at 1.34 µM while supplemented with the same volume of acetone was used as negative control. Cells were monitored for up to 36 h at 28 °C at 2 × 1 psi flow rate. Phase contrast images were acquired and processed as previously described.

In total, 1264 cells were analysed and all experiments were performed in three biological replicates.

Biofilm formation assay. Biofilm formation of Phaeobacter inhibens and S. dubsia was analysed by a crystal violet assay. P. inhibens and S. dubsia were grown in 10 mL MB medium (MB, Carl Roth) at 28 °C with vigorous shaking to the early exponential phase (OD600 of 0.4). Cultures were subsequently diluted to an OD600 of 0.065. 100 µL of the diluted culture was transferred to a respective well of a sterile polystyrene 96-well plate (Corning, New York, NY, USA; Costar 3370). Two hundred microlitre of 0.5% crystal violet (CV) was added to the wells, which were incubated at room temperature for 10 min. After staining, the CV solution was removed and each well was washed twice with H2O to remove the residual dye and dried overnight. For scoring of cell attachment, the CV was extracted from the biofilm with 200 µL 95% (v/v) ethanol, of which 100 µL were transferred to a new 96-well plate before the absorbance was determined at 595 nm using the Tecan microplate reader (Infinity® 200 PRO). For each experiment, four biological replicates and two technical replicates were performed.

Analysis of the stieleriacine production rate. To analyse the production rate of stieleriacine A1, precultures of strain Mal15 were inoculated 1:10 in fresh M1H NAG ASW medium and incubated for 7 days (28 °C, 80 rpm). For the next five days 30 mL of each culture were harvested every 12 h, followed by 2 days harvesting after every 16 h. The harvested material was analysed for optical density (OD600), biomass and stieleriacine A1 concentration.

Time-lapse microscopy. To analyse the physiological effect of stieleriacine A1, with time-lapse microscopy, a preculture of Mal15 cells was inoculated 1:10 in fresh M1H NAG ASW medium and incubated for 72 h (28 °C, 80 rpm). Before loading, the CellASIC® microfluidic chamber was washed with a constant flow of medium (2 × 5 psi, 5 min). Stieleriacine A1 was provided dissolved in 132 µL acetone at 1.34 µM, while supplemented with the same volume of acetone was used as a negative control. Cells were monitored for up to 36 h at 28 °C at 2 × 1 psi flow rate. Phase contrast images were acquired and processed as previously described.

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Lee, I., Ouk Kim, Y., Park, S. C. & Chun, J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int. J. Syst. Evol. Microbiol.* **66**, 1100–1103 (2016).

Rodriguez-R, L. M. & Konstantinidis, K. T. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Prepr.* **4**, e1900v1 (2016).

Qin, Q.-L. et al. A proposed genus boundary for the prokaryotes based on genomic insights. *J. Bacteriol.* **196**, 2210–2215 (2014).

Bondoso, J., Harder, J. & Lage, O. M. rpoB gene as a novel molecular marker to infer phylogeny in *Planctomycetaceae*. *Antonie van Leeuwenhoek* **104**, 477–488 (2013).

Sievers, F. et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**, 539 (2011).

Surup, F. et al. Deconins A–E: cupenic acid and mevalonic or propionic acid conjugates from the basidiomycete *Deconica* sp. **471**, *J. Nat. Prod.* **78**, 934–938 (2015).

Boeckers, C. et al. Determining the bacterial cell biology of planctomycetes. *Nat. Commun.* **8**, 14853 (2017).

O’Toole, G. A. & Kolter, R. Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Mol. Microbiol.* **28**, 449–461 (1998).

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**Author contributions**

N.K. wrote the final version of the manuscript with help of C.J., based on an initial version written by O.J., B.S. and F.S. O.J. performed most biological experiments. N.K. analysed the genome and proposed the biosynthesis pathway with help from M.H.M. B.S. performed most of the chemical experiments with the help of F.S. C.B. performed time-lapse and light microscopy experiments. S.W. sequenced and characterised the genome and performed the phylogentic analysis. P.B. performed *Roseobacter*-related experiments and constructed the mutants with help from J.P. M.J. developed the novel isolation strategy with help from C.J., isolated the strain and performed general microbiological experiments. M.R. took electron micrographs. J.P. designed the *Roseobacter*-related experiments and supervised P.B. M.H.M supported N.K. and both analysed genes related to small molecule production. F.S. supervised B.S. and guided structure elucidation. C.J. and F.S. designed the concept of this study. C.J. supervised O.J. All authors read and approved the final version of the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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