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1 Methods

1.1 Chemicals & Instruments

All chemicals and solvents were supplied by Merck, if not specified otherwise. Vendors for specific solvents are listed in Table 1.1. Water was purified prior to use by a MilliQ filtration system.

Table 1.1: Used chemicals and solvents

Chemical	**Supplier**
J. T. Baker	Acetonitrile
	Chloroform
Alfa Aesar	Methyl acetate
Fisher Chemicals	Ethyl acetate

High performance liquid chromatography (HPLC) systems were manufactured by Agilent. The components of the HPLC systems are listed in Table 1.2. Detailed specifications of used HPLC-columns are listed in Table 1.3.

Table 1.2: Components of HPLC systems

	Component	**Description**
Agilent 1100 Series	G1322A	Degasser
	G1311A	Quaternary Pump
	G1313A	Autosampler
	G1316A	Column Compartment
	G1315B	Diode Array Detector
Agilent 1200 Series	G1379B	Degasser
	G1312A	Binary Pump
	G1367B	Autosampler
	G1330B	Thermostat
	G1316A	Column Compartment
	G1315B	Diode Array Detector
Agilent 1260 Infinity	G4225A	Degasser
	G1312C	Binary Pump
	G1329B	Autosampler
	G1330B	Thermostat
	G1316A	Column Compartment
	G1315D	Diode Array Detector

Table 1.3: Column Specifications

Manufacturer	**Line**	**Type**	**Size**
Merck	SeQuant®	ZIC®-HILIC 3.5 µm	150 × 4.6 mm
Phenomenex	Luna®	NH2 5 µm	250 × 4.6 mm
	Kinetex®	Polar-C18 2.6 µm	150 × 4.6 mm
Dr. Maisch	Nucleosil-100	C18 5 µm	100 × 2.5 mm

1.2 Strain Cultivation

1.2.1 Media

All media were prepared by solving the components listed in Table 1.4 in MilliQ-H_2O and adjusting the pH with NaOH and HCl. For solid media, 2 % (w/v) agar was added. Media were sterilized by autoclaving at 121 °C and 230 kPa for 15 min. Fluid media were stored at ambient temperature, solid media at 8 °C.

1.2.2 *Escherichia coli* K12 and *Bacillus subtilis* 168

Escherichia coli K12 and *Bacillus subtilis* 168 were cultivated in LB medium (10 g peptone, 5 g yeast extract, 10 g NaCl per liter; pre-mixed by Roth) at either 37 °C (K12) or 30 °C (168). Liquid cultures were shaken at 200 rpm in flasks with baffles and spirals. Plate cultures were grown in an incubator.

1.2.3 General cultivation of *Streptomyces* sp. Tü2401

Agar-plate cultures of *Streptomyces* sp. Tü2401 were grown on ISP2 medium at 29 °C. 100 µL of spore solution or liquid culture were used for inoculation. Liquid cultures were incubated at 27 °C in shake flasks with aluminium caps. Pre-cultures were grown in 20 mL of NL 410 in 100 mL flasks and inoculated with plate-grown mycelium. Main cultures were grown in 100 mL medium in 500 mL flasks.

1.2.4 Batch Fermentation of *Streptomyces* sp. Tü2401

Streptmyces sp. Tü2401 was cultivated at a ten-liter scale in a continuous stirred tank bioreactor. 500 mL of pre-culture were grown in five 500 mL shake flasks containing 100 mL of NL 410 medium without $CaCO_3$. The pre-cultures were inoculated from stored ISP-agar plates and grown for 72 h at 27 °C. The pre-cultures were pooled and used to inoculate 9.5 L of OM medium for fermentation. The temperature was kept at 27 °C with an airflow of 5 L min^{-1} and a rotor speed of 200 rpm. Control samples of 15 mL were taken throughout the process at regular intervals. Fermentation was stopped after 125 h and the culture broth was harvested. Further processing is described in section 1.4.3.

Table 1.4: **Media components for the cultivation of strain Tü2401.** All amounts are calculated for one liter of MilliQ-H_2O. The pH was adjusted with NaOH and HCl.

Name	pH	Component	Amount	Vendor
LB		Yeast extract	5 g	Roth
		Tryptone	10 g	Roth
		NaCl	10 g	Roth
ISP2	7.3	Yeast extract	4 g	Oxoid
		Malt extract	10 g	Thermo Fisher
NL 200	7.5	D(-)Mannitol	20 g	Merck
		Cornsteep Powder	20 g	Sigma-Aldrich
NL 300	7.5	D(-)Mannitol	20 g	Merck
		Cotton Seed	20 g	Pharmamedia
NL 410	7.0	Glucose	10 g	Roth
		Glycerol	10 g	Acros Organics
		Oatmeal	5 g	Holo Bio Hafergold
		Soymeal	10 g	Hensel
		Yeast extract	5 g	Oxoid
		Bacto Casaminoacids	5 g	Difco
		$CaCO_3$	1 g	
NL 500	8.0	Starch	10 g	Roth
		Glucose	10 g	Roth
		Glycerol	10 g	Acros Organics
		Fish Meal	15 g	Sigma-Aldrich
		Sea Salts	10 g	Sigma-Aldrich
OM	7.3	Oatmeal	20 g	Holo Bio Hafergold
		Trace metal mix	5 mL	
Trace metal mix		$CaCl_2 \cdot 2\,H_2O$	3 g	
		Fe^{3+} citrate	1 g	
		$MnSO_4 \cdot H_2O$	200 mg	
		$ZnCl_2$	100 mg	
		$CuSO_4 \cdot 5\,H_2O$	25 mg	
		$Na_2B_4O_7 \cdot 10\,H_2O$	20 mg	
		$CoCl_2 \cdot 6\,H_2O$	4 mg	
		$Na_2MoO_4 \cdot 2\,H_2O$	10 mg	

1.3 Bioassays

1.3.1 Agar Diffusion Bioactivity Assays

Agar diffusion bioactivity assays against *E. coli* K12 and *B. subtilis* 168 were conducted on LB-agar in petri dishes. Round petri dishes ($\varnothing = 92\,\mathrm{mm}$) were filled with 20 mL of liquid agar, square dishes ($120 \times 120\,\mathrm{mm}$) were filled with 40 mL. Solidified agar plates were stored at 8 °C.

400 µL (200 µL for round plates) of liquid culture at an optical density of 0.3 to 0.6 at 600 nm were spread on the solid agar plate with a drigalski spatula until dry. Round wells ($\varnothing = 7\,\mathrm{mm}$) were punched out of the agar and filled with 50 µL of sample. Finished plates were stored for 1 h at ambient temperature, before incubating them at either 30 °C or 37 °C over night.

1.3.2 *yorB* Reporter Gene Assay

Agar-based *yorB*-reporter gene assays were performed with *Bacillus subtilis* 1S34 pHJS105-yorB-lacZ2.[1] 20 mL of LB-agar with added $50\,\mu\mathrm{g\,mL^{-1}}$ Spectinomycin were inoculated with 500 µL of overnight culture and grown until the stationary phase. 50 mL LB-softagar (0.7 % agarose) with $150\,\mu\mathrm{g\,mL^{-1}}$ 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were prepared. The strain was added to a concentration of 3×10^7 colony-forming units per milliliter and poured into a square petri-dish. After solidification, 50 µL of test samples were filled in wells. The plates were incubated at 30 °C for 14 to 18 h and analyzed for induction.

1.4 Sample Preparation and Extraction

Extracts and reverse extracts of *Streptomyces* sp. Tü2401 were generally obtained through filtrated medium supernatants. After cultivating the strain for 4 to 7 days, the harvested biomass was centrifuged at 9000 rpm for 20 min. The supernatant was collected and filtered through a 0.2 µm sterile filter. The filtrate was stored at 4 °C.

1.4.1 Preparation of medium extracts

Medium filtrate extracts were prepared by adding an equal amount of solvent to the filtrate and shaking the mixture for 30 min. The phases were separated by centrifugation at 4000 rpm for 10 min. Both phases were collected separately and stored at 4 °C. Samples were concentrated by drying them under vacuum at either 40 °C (Ethyl acetate, Methyl acetate) or 60 °C (Butanol, water) and resuspending them in a fifth of the initial volume.

1.4.2 Determination of Extraction Conditions

5 mL filtered medium aliquots of Tü2401 were transferred to three sets of five 15 mL falcon tubes. The pH of the tubes in each set was adjusted to 2, 5, 7, 9 or 11 with NaOH and HCl. Each set was extracted with either ethyl acetate, methyl acetate or ethyl formate and both phases were collected. Each phase was tested for bioactivity against *E. coli* K12.

1.4.3 Processing of Fermentation Broth

The harvested fermentation broth was supplemented with diatomaceous earth and filtered through Pall T 1500 filter plates (relative retention range 10 - 30 µm). The remaining filter cake was discarded and the filtrate transferred to a stirring bucket . Two liters of ethyl acetate were added to the filtrate and stirred for 30 min. After completed phase-separation, the organic phase was collected and the aqueous phase reused for further extraction. The process was repeated five times. Both phases were collected seperately and concentrated in a rotary evaporator at 40 °C.

Englischen Begriff nachsehen

1.4.4 Agar Plate Extraction

Standard ISP2 agar plates were ground with a blender and extracted with with an equal volume of butanol or ethyl acetate for 1 h. The mixture was centrifuged at 4000 rpm for 1 h and the supernatant collected. The remaining slurry was resuspended in the same amount of water, centrifuged at 4000 rpm for 1 h and the supernatant collected.

Special ISP2 agar plates with low-melting-point agarose (LMPA) were prepared by substituting the 2 % (w/v) agar of regular plates with 4 % (w/v) LMPA. 75 mL of LMPA agar plates were melted in Schott-flasks at 70 °C and extracted with either butanol or ethyl acetate for 30 min. The organic phase was collected and the remaining agar extracted again with 50 mL of water.

All collected organic extracts were dried at 40 °C (ethyl acetate) or 60 °C (butanol) and resuspended in 1 mL methanol.

1.5 Bioactivity-guided Isolation

1.5.1 Thin Layer Chromatography

Thin layer Chromatography was performed with reverse extracts of Tü2401 on TLC Silica Gel 60 F_{254} plates by Merck. Aqueous samples were applied by pipetting 1 µL at a time and letting the plate dry until the next application. The TLC chambers were filled up to 1 cm with solvent and incubated for 12 h. The plates were run until either 75 % of the plate had been soaked or 2 h had passed. The solvents used as mobile phases are listed in Table 1.5.

Table 1.5: **Mobile phase compositions used for Thin-Layer Chromatography**

Solvent	**Ratio (v/v)**
Acetonitrile / Water	85:15
Butanol / Acetic acid / Water	14:3:2 and 42:10:7
Butanol / Ethanol / Water	3:2:1
Ethyl acetate / 2-Propanol / Water	6:3:1
Chloroform / Methanol	8:3

The working orcin staining solution was prepared by mixing two storage solutions, solution A and B, at a ratio of 10:1 (v/v). Solution A contained 1 % (w/v) $Fe^{III}Cl$ in 10 % sulfuric acid, solution B contained 6 % (w/v) Orcin in ethanol. The plates were sprayed with the working solution and treated with a heat gun for a few seconds.

Preparative samples were obtained by scraping the silica off the unstained plate and collecting it in reaction tubes. The samples were then extracted with 1 mL methanol, vortexed and sonicated for 30 min. After centrifugation at 14,000 rpm for 5 min, the supernatant was transferred to a new tube and the extraction process repeated. The methanolic samples were dried at 30 °C and resuspended in an amount of water equal to the sample initially applied on the TLC plate.

1.5.2 Ion Exchange Chromatography

Ion exchange chromatography was performed with both a strong anion (Diaion SA11A, 20-50 mesh, Cl^- form) and a strong cation exchange resin (Dowex 50WX4, 100-200 mesh, Na^+ form). Three solutions were used for all operations: An acidic solution (1 % (v/v) formic acid, pH 2), a neutral solution (MilliQ-H_2O, pH 7) and a basic solution (2 % (v/v)

ammonium hydroxide, pH 11). Prior to column preparation, both resins were swollen for 24 h. The anion exchange resin (AnX) was swollen in the basic solution and the cation exchange resin (CatX) was swollen in the acidic solution. 12.5 mm diameter glass columns were filled with resin up to a bed height of 10 cm (AnX) or 9.5 cm (CatX). Both columns were operated at a constant flow of 2.5 mL min^{-1}. All method steps are listed in Table 1.6. The pH of the applied sample was adjusted to pH 2 (CatX) or pH 11 (AnX) with NaOH and HCl. The flow-through of each step was collected and stored at 4 °C.

Table 1.6: **Method for ion exchange chromatography.** pH values and relative volume of the solutions used for ion exchange chromatography with both strong anion exchange (AnX) and cation exchange (CatX) resins. Both resins were loaded with 1 mL of sample

Step	AnX pH	CatX pH	Column Volumes
Equilibration	11	2	2
Wash 1	7	7	1
Sample application	11	2	*
Wash 2	11	2	1
Wash 3	7	7	1
Elution	2	11	5

1.5.3 Trimethylsilane Derivatization and Gas Chromatography

The derivatization and gas chromatography (GC) measurements were performed by Dr. Dorothee Wistuba of the mass spectrometry department at the institute of organic chemistry. Dried HPLC fractions were suspended in 500 µL *N,O*-Bis(trimethylsilyl)trifluoroacetamide with added 40 µL pyridine and heated to 110 °C. Afterwards, the samples were dried with nitrogen gas and redissolved in dichloromethane. The derivatized samples were analyzed with a Hewlett Packard (HP) 6890 GC-system coupled to a HP 5973 mass selective detector. The Agilent DB5 column measured 13 m × 0.25 mm with a film thickness of 0.1 µm. Helium was used as the carrier gas.

1.5.4 Preparative HPLC

Preparative HPLC was performed on either the Agilent 1100 Series or Agilent 1260 Infinity instrument coupled to an Agilent fraction collector. Occasional coupling of an electric light

Add instrument number of FC

scattering detector (ELSD) was achieved by mounting a 1:5-splitter after the UV-Detector. 4/5 of the flow was directed to the fraction collector, the remaining 1/5 was directed to the ELSD. The ELSD was operated at a temperature of 40 °C and with nitrogen gas at a pressure of 3.2 bar. Detailed information about the used columns and methods can be found in Table 1.3 and the appendix. The obtained data was analyzed with the Agilent Chemstation (Version B.04.03). All samples were centrifuged at 14,000 rpm, before transferring the supernatant to HPLC-vials. All fractions were collected by timeslices of 1 min and stored at 4 °C to 8 °C, before drying them at 40 °C. Dried fractions were resuspended in an amount of water equal to the amount of injected sample and stored at 4 °C to 8 °C.

1.5.5 Analytical HPLC and Mass Spectrometry

For mass spectrometry, an Agilent 1200 series HPLC system was coupled to an Agilent 6330 IonTrap LC-MS mass spectrometer. It features electrospray ionization with alternating positive and negative modes. The control software was 6300 Series Trap Control (Version 6.1).

1.6 Genome Analysis

1.7 AntiSMASH Cluster Analysis

All five contigs were uploaded to the AntiSMASH biosynthetic gene cluster identification tool[2–4]

1.8 Phylogenetic determination

2 Results & Discussion

2.1 Determination of Extraction Conditions

2.2 Chromatographic Separation

2.2.1 Reverse-Phase HPLC

2.2.2 HPLC with Amino Column

2.2.3 Hydrophilic Interaction Chromatography

2.2.4 Ion exchange Chromatography

2.2.5 Thin-Layer Chromatography

2.3 Dereplication

2.3.1 HPLC Mass Spectrometry

2.3.2 Trimethylsilane Derivatization and Gas Chromatography

2.4 Antibacterial Activity Spectrum

2.4.1 Activity against E. coli

2.4.2 Activity against B. subtilis

2.4.3 Extraction of yorB-inducing Compound

Tü2401 displayed positive results in the yorB-Assay when grown on ISP2 plates . However,

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all previously generated samples from liquid cultures only showed antibacterial activity. Three cultivation strategies were developed to induce production of the putative compound 2 (PC2):

1. Standing cultures could allow the formation of aerial mycelium at the medium surface. Thus, enabling the synthesis of PC2 and allowing extraction of the liquid medium. Two 500 mL flasks were each filled with 100 mL of liquid ISP2 medium and inoculated with 1 mL of a one-week old NL 410 shake culture. One flask was sealed with an ordinary aluminium cap (AC), the other one with an air-permeable foam cap (FC). Both flasks were cultivated for four days, before the medium was centrifuged and the supernatant filtrated. 50 mL aliquots of each flask were extracted with either BuOH or EtAc, and both phases were collected seperately. The organic phases were dried at 40 °C and solved in 1 mL MeOH. Both phases of each flask were subjected to the yorB-Assay.

2. ISP2 agar plates were previously known to enable synthesis of PC2 and could be extracted with prior breakup. Ten round ISP2 agar plates were inoculated with 100 µL of a one-week old NL 410 shake culture and incubated for four days. One half of the plates was extracted with BuOH, the other half was extracted with EtAc.

3. ISP2 agar plates could also be prepared with low-melting-point agarose (LMPA). This would allow melting of the plates at lower temperatures. Thus, allowing for easier extraction with reduced risk of thermal decomposition during the process. Six ISP2 LMPA agar plates were inoculated with 100 µL of a week-old NL 410 shake culture and incubated for four days. The plates were then melted at 70 °C and extracted with BuOH and EtAc.

All generated extracts and their respective aqueous phases were tested for bioactivity. The standard bioassay was used to determine the antibiotic activity against *E. coli* K12 and *B. subtilis* 168, and the yorB-Assay was used to test for the mode of action. Additonally, agar stamps of grown ISP2 and ISP2 LMPA plates were tested in the yorB-Assay. A summary of the results is shown in .

Tabelle einsetzen

Table 2.1: **Bioassay results from agar-plate and standing culture extraction**. Samples were screened for antibiotic activity against *E. coli* K12 and *B. subtilis* 168 and for promotor induction in the yorB-Assay. Samples from standing cultures with foam cap (FC) or aluminium cap (AC), ISP2 agar plates (ISP2) and ISP2 agar plates with low-melting-point agarose (LMPA). Samples were extracted with butanol (BuOH) or ethyl acetate (EtAc) and tested alongside their respective aqueous phases (aq.). *Legend*: **-** No activity; **+ / ++ / +++** antibiotic activity with inhibition zone of 1 / 1.0-1.5 / >1.5 cm; **n.e.** result non-evaluable

	Antibacterial			positive
Sample	***E. coli***	***B. subtilis***	**yorB**	**yorB**
FC BuOH	-	-	-	-
FC BuOH aq.	-	-	-	-
FC EtAc	-	+++	++	-
FC EtAc aq.	-	-	-	-
AC BuOH	-	-	-	-
AC BuOH aq.	-	-	-	-
AC EtAc	-	-	+	-
AC EtAc aq.	-	-	-	-
ISP2 BuOH	-	n.e.	+++	-
ISP2 BuOH aq.	-	+	-	-
ISP2 EtAc	-	++	-	-
ISP2 EtAc aq.	+	n.e.	-	+
ISP2 plaque			++	++
LMPA BuOH	+	n.e.	+++	-
LMPA BuOH aq.	-	n.e.	-	-
LMPA EtAc	-	n.e.	+++	-
LMPA EtAc aq.	-	n.e.	-	-
LMPA plaque			++	++

2.5 Genomic Analysis

2.5.1 Phylogeny of Strain Tü2401

2.5.2 AntiSMASH Cluster Identification

One cluster was detected on contig four and identified as a Type1-PKS-NRPS hybrid. It shows a 95 % cluster identity to the C-1027 biosynthetic gene cluster from *Streptomyces* globisporus C-1027 (MiBiG accession no. BGC0000965). Additionally, three homologous subclusters with 100 % identity were identified, which are associated with the synthesis of C-1027, Neocarzinostatin and Maduropeptin enediynes (see Figure .1).The presence of this cluster could indicate, that the strain Tü2401 is capable of producing a compound similar to enediyne antibiotics.

Enediyne natural products are a class of cytotoxic bacterial compounds, which cause extensive DNA-damage.[5–8] 11 different enediyne natural products are known, all of which feature either a bicyclo[7.3.0]dodecadienediyne core inside a nine-membered ring or a bicyclo[7.3.1]tridecadiynene core inside a ten-membered ring (Figure **??**). The 9-membered family includes neocarzinostatin, C-1027 and and maduropeptin. The 10-membered family includes calicheamicin γ_1^I, esperamicin A_1 and dynemicin A.[5] Enediynes are potent cytotoxic agents because of their ability to induce DNA double-strand breaks.[9] Electronic rearrangement of the carbocycle produces a benzenoid diradical, which abstracts hydrogen atoms from the DNA-backbone. The consulting radicals cause interstrand crosslinks or react with molecular oxygen. While the ten-membered enediyne compounds were isolated as free-standing chromophores, most of the compounds in the nine-membered family were isolated in conjunction with a protective apoprotein.[5] The nine-membered chromophore of C-1027, which was isolated from *Streptomyces* globisporus C-1027, is bound noncovalently to an 110 amino acid apoprotein.[7,10–17] The isolated chromophore has been shown to be very unstable, whereas the holo C-1027 did not lose activity under the same conditions.[13–15] The apoprotein binds specifically to the C-1027 chromophore, supposedly by hydrophic pocket, which binds the benzoxazine side chain.[14,18] The enediyne antibiotics neocarzinostatin and maduropeptin, which were also isolated from actinomycetes, also feature highly specific and protective apoproteins..[7]

The homologies of the identified cluster could be an indicator, that the strain Tü2401 is capable of synthesizing an enediyene antibiotic with a nine-membered core and a corre-

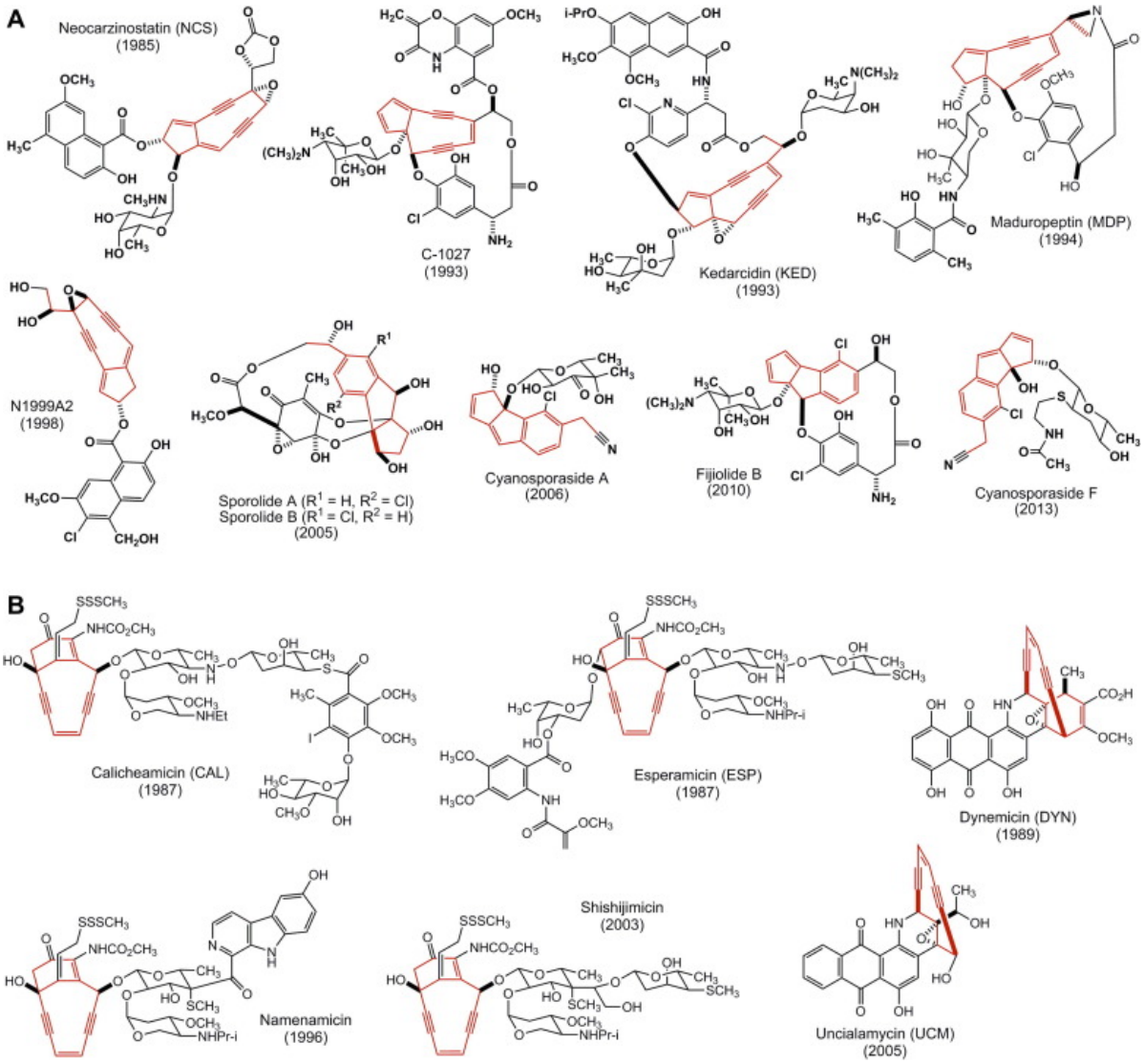

Figure 2.1: **Structures of known enediyne natural products.** Enediyne cores are highlighted in red. (A) Compounds with nine-membered rings. (B) Compounds with ten-membered rings. The year of structure confirmation is displayed in parentheses. The sporolides, cyanosporolids and fijiolides do not contain an endiyene core, but are proposed to be derived from enediyne precursors. Reprinted from Shen *et al.* (2015) Copyright 2014 Elsevier Ltd.

sponding apoprotein. The potent DNA-strand-breaking capabilities of this compound-class could induce the *yorB* reporter system of *Bacillus subtilis* 1S34 pHJS105-yorB-lacZ2 in the yorB-induction assay. A number of compounds, which cause DNA double strand breaks and crosslinks are reported to induce the system, though none of them belong to the family of endiyne antibiotics.[1] Whether this is due to inactivity or to this family not having been tested in the assay is unclear, since the compound library is not publicly accessible. Nevertheless, an endiyne compound could be responsible for the induction.

The assay in Section 2.4.3 showed, that the *yorB* inducing compound is produced when the strain is grown on an ISP2 agar plate, but it could not be extracted via ethyl acetate or butanol. Only very low activity was retained in the aqueous phase. If the compound is indeed an endiyne, this loss of activity could be due to the high instability of the chromophore. The apoprotein could have been detached during the extraction and concentration process, which also included high temperatures of 40 to 60 °C. Combined with an incubation time of several days between extraction and assay, this could have led to the degradation of the chromophore below the sensitivity threshhold. The high temperatures and long storage times also apply to the numerous HPLC-fractioning samples subjected to the *yorB*-induction assay.

To verify this assumption, the putative endiyne compound has to be extracted by an adapted protocol, dereplicated and subjected to the assay in pure form. The C-1027 antibiotic protein from *S. globisporus* was precipitated from the medium supernatant by the addition of ammonium sulfate and purified by dialysis and column chromatography.[16] The active chromophore could be extracted from the apoprotein with methanol at 0 °C.[14] However, as of now, untreated medium supernatant samples of the strain Tü2401 did not induce the *yorB*-reporter system. The cultivation in liquid OM medium is probably not sufficient for production of the putative endiyene compound and the protocol would have to be adapted for the extraction from ISP2 agar plates. To circumvent this, other cultivation media could be employed and tested for *yorB*-induction. The holo endiyne-apoprotein complex should be stable enough for routine testing of media and media supernatants. The only alternative to optimizing production conditions would be heterologous expression of the biosynthetic cluster. For the endiyne compound family though, this has, as of now, only been partly achieved for the nine-membered endiyne neocarzinostatin.[19]

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A Appendix

HPLC Methods

Vielleicht wenn hier text steht

Table A.1: **Standard Screening Method**

Parameter	**Value**
Column	Nucleosil-100 C18 5 µm 150×3 mm
Solvents	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.1 % Formic acid
Method	Gradient 5 - 100 % B for 15 min
	Plateau 100 % B for 3 min
Flow	1.25 mL min^{-1}
Temperature	25 °C
Injection Volume	50 µL

Table A.2: **Standard aminocolumn method**

Parameter	**Value**
Column	Luna NH2 5 µm 250×4.6 mm
Solvents	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.1 % Formic acid
Method	Isocratic 80 % B for 20 min
	+ 100 % A for 10 min
Flow	2 mL min^{-1}
Temperature	25 °C
Injection Volume	50 µL

Table A.3: **The standard HILIC method**

Component	**Parameter**
Column	ZIC-HILIC 3.5 µm 150×4.6 mm
Solvents	A: 10 mM Ammonium acetate
	B: Acetonitrile
Method	Isocratic 80 % B for 45 min.
Flow	0.8 mL min^{-1}
Temperature	25 °C
Injection Volume	50 µL

Table A.4: **HILIC method adapted for MS coupling**

Component	**Parameter**
Column	ZIC-HILIC 3.5 µm 150×4.6 mm
Solvents	A: 10 mM Ammonium acetate
	B: Acetonitrile
Method	Isocratic 80 % B for 60 min.
Flow	0.5 mL min^{-1}
Temperature	40 °C
Injection Volume	50 µL

Table A.5: **Screening method for HPLC-MS**

Parameter	**Value**
Column	Nucleosil-100 5 µm 150×3 mm
Solvents	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.06 % Formic acid
Method	Gradient 0 - 100 % B for 15 min
	Plateau 100 % B for 2 min
Flow	0.4 mL min^{-1}
Temperature	40 °C
Injection Volume	2.5 µL
Capillary Voltage	3500 V
Injector Temperature	350 °C
Target mass	400 m/z

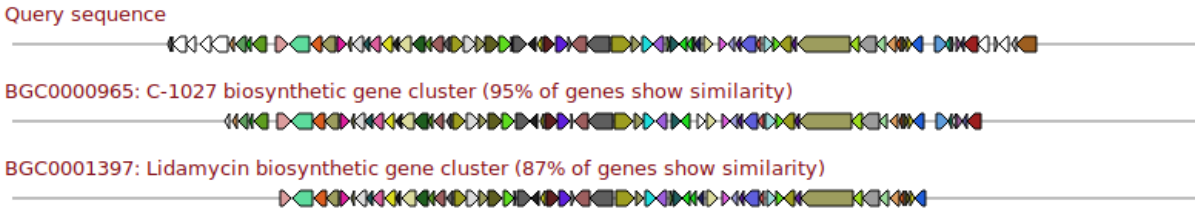
Table A.6: **Screening Method Polar-C18**

Parameter	**Value**
Column	Kinetex Polar-C18 2.6 µm 150×4.6 mm
Solvents	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.1 % Formic acid
Method	Gradient 5 - 100 % B for 20 min
	Plateau 100 % B for 6 min
Flow	1.2 mL min^{-1}
Temperature	50 °C
Injection Volume	50 µL

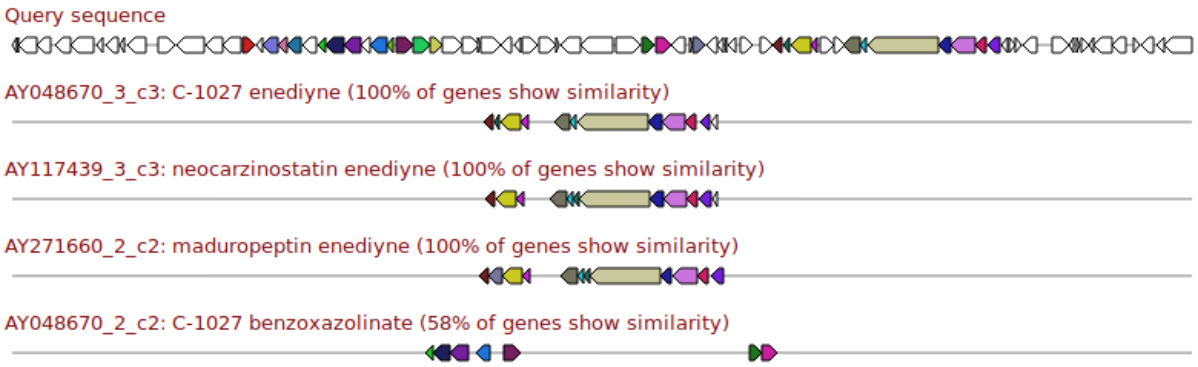
Table A.7: **Reverse Screening Method Polar-C18**

Parameter	**Value**
Column	Kinetex Polar-C18 2.6 µm 150×4.6 mm
Solvents	A: Water + 0.1 % Formic acid
	B: Acetonitrile + 0.1 % Formic acid
Method	Gradient 100 - 5 % B for 20 min
	Plateau 100 % B for 6 min
Flow	1.2 mL min^{-1}
Temperature	50 °C
Injection Volume	50 µL

Genomic analysis



(a) Cluster search results for the identified cluster.



(b) Subcluster search results for the identified cluster.

Figure A.1: **Cluster and subcluster search results for the cluster located on contig four.** The 160 kb contig was submitted to AntiSMASH with the ClusterFinder option. Only the search results with the highest similarities are shown.