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# 1 Methods

## 1.1 Chemicals & Instruments

Chemicals and solvents were supplied by Merck, if not specified otherwise. Differing vendors for solvents are listed in Table 1.1

**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.

**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 Parameters

Manufacturer	Line	Type	Size
Merck	SeQuant <sup>®</sup>	ZIC <sup>®</sup> -HILIC PEEK 3.5 $\mu$ m	150 $\times$ 4.6 mm
Phenomenex	Luna <sup>®</sup>	NH2 5 $\mu$	250 $\times$ 4.6 mm
	Kinetex <sup>®</sup>	Polar-C18 2.6 $\mu$ m	150 $\times$ 4.6 mm
Dr. Maisch	Nucleosil-100	C-18 5 $\mu$ m	100 $\times$ 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<sub>2</sub>O and adjusting the pH with NaOH and HCl. For solid media, 2 % (w/v) agar was added. Sterilization was performed by autoclaving at 121 °C and 100 000 kPa for 15 min. Fluid media were stored at ambient temperature, solid media at 8 °C.

### 1.2.2 General cultivation of *Streptomyces* sp. Tü2401

*Streptomyces* sp. Tü2401 was cultivated primarily in liquid NL- or OM-medium. Agar-plate cultures 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.

### 1.2.3 *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 generated by adding 2 % (w/v) agar to the medium.

### 1.2.4 Batch Fermentation

The strain 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 round flasks containing 100 mL of NL 410 medium without CaCO<sub>3</sub>. 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 NL OM medium for fermentation. The temperature was kept at 27 °C with an airflow of 5 L min<sup>-1</sup> 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 1.3.1.

**Table 1.4: Media components for the cultivation of strain Tü2401.** All amounts are calculated for one liter of MilliQ-H<sub>2</sub>O. 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 <sub>3</sub>	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 <sub>2</sub> · 2 H <sub>2</sub> O	3 g	
		Fe <sup>3+</sup> citrate	1 g	
		MnSO <sub>4</sub> · H <sub>2</sub> O	200 mg	
		ZnCl <sub>2</sub>	100 mg	
		CuSO <sub>4</sub> · 5 H <sub>2</sub> O	25 mg	
		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> · 10 H <sub>2</sub> O	20 mg	
		CoCl <sub>2</sub> · 6 H <sub>2</sub> O	4 mg	
		Na <sub>2</sub> MoO <sub>4</sub> · 2 H <sub>2</sub> O	10 mg	

## 1.3 Sample Preparation

### 1.3.1 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  $\mu\text{m}$ ). 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 separately and concentrated in a rotary evaporator at 40 °C.

## 1.4 Chromatographic Methods

### 1.4.1 Thin Layer Chromatography

Thin layer Chromatography was performed with reverse extracts of Tü2401 on TLC Silica Gel 60 F<sub>254</sub> plates by Merck. Aqueous samples were applied by pipetting 1  $\mu$ L 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<sup>III</sup>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.

### 1.4.2 Ion Exchange Chromatography

Ion exchange chromatography was performed with both a strong anion (Diaion SA11A, 20-50 mesh, Cl<sup>-</sup> form) and a strong cation exchange resin (Dowex 50WX4, 100-200 mesh, Na<sup>+</sup> form). Three solutions were used for all operations: An acidic solution (1 % (v/v)

formic acid, pH 2), a neutral solution (MilliQ-H<sub>2</sub>O, 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<sup>-1</sup>. 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

### 1.4.3 Time-based fractionation by HPLC

Culture extracts of strain Tü2401 were fractioned via HPLC coupled to an automated fraction collector.

Hydrophilic Interaction Chromatography (HILIC) with performed with a 4,6 x 250 mm ZIC-HILIC Column (Merck). It features zwitterionic, functional groups on poly(etherether ketone) (PEEK) material. 10 mM Ammonium acetate in Milli-Q H<sub>2</sub>O was used as solvent A, while Acetonitrile comprised solvent B. Detailed method descriptions regarding solvent composition, flow and duration are listed in the appendix.

### 1.4.4 Analytical HPLC and Mass Spectrometry

Obtained



### **1.4.5 Mass Spectrometry**

A test table should be here