# List of Tables

# 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® | ZIC®-HILIC PEEK 3.5 µm | 150 × 4.6 mm |
| Phenomenex | Luna® | NH2 5u | 250 × 4.6 mm |
| | Kinetex® | Polar-C18 2.6 µm | 150 × 4.6 mm |
| Dr. Maisch | Nucleosil-100 | C-18 5 µm | 100 × 2.5 mm |

## 1.2 Strain Cultivation

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

*Streptomyces* sp. Tü2401 was cultivated in a variety of complex media, which are listed in Table 1.4. 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.

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

### 1.2.3 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 $CaCO_3$. 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 NL 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 1.3.1.

### 1.2.4 Media

The used media are listed in Table 1.4

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

| Name | pH | Component | Amount | Vendor |
|---|---|---|---|---|
| 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 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 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.

## 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 $F_{254}$ 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.

### 1.4.2 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 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 Hydrophilic Interaction Chromatography

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 $H_2O$ 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 High Performance Liquid Chromatography

### 1.4.5 Mass Spectrometry

A test table should be here