

**Isolation of a hydrophilic, antibacterial compound from
strain *Streptomyces* Tü2401**

**Masterarbeit
der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen**

**vorgelegt von
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Tübingen, September 2016**

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Zusammenfassung

Abstract

Danksagung

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1 Introduction

1.1 First part

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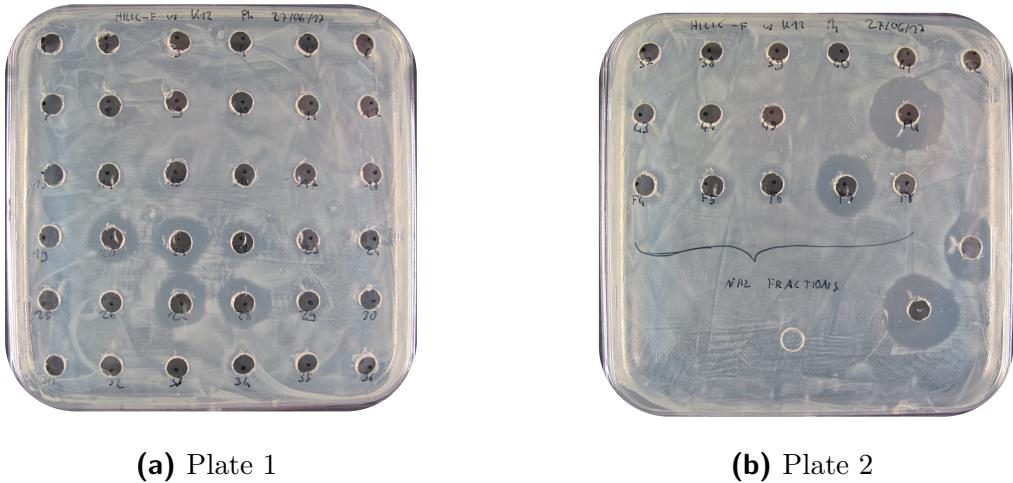


Figure 1.1: Plate assay of HILIC fractions against K12. asdjj sdj sdjkfj sfsd sjjfkdjf lskfjsd askjs s djfkd s ls s kj sdkjfksjf als slkd s

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Table 1.1: Parameters of Merck ZIC-HILIC Column

Manufacturer	Line	Type	Dimensions
Merck	SeQuant®	ZIC-HILIC	150 × 4.6 mm
Phenomenex	Luna®	NH2	250 × 4.6 mm
Dr. Maisch	Nucleosil-100	C-18	100 × 2.5 mm

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1 Introduction

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2 Methods

2.1 Chemicals & Instruments

Chemicals

The used instruments are listed in table 2.1.

Table 2.1: Used laboratory Instruments

Instrument	Model	Manufacturer
Centrifuges	Megafuge 1.0 (R) Centrifuge 5417 C RC6 Plus Centrifuge	Heraeus Instruments Eppendorf Sorvall
Lyophilizator	LyoVac GT2	Leybold
Spectrophotometer	BioMate 3S	Thermo Fisher
Rotary Evaporator	Hei-Vap Precision Rotavapor RE + PC 3001 VARIO Pump	Heidolph Büchi + vacuubrand

High performance liquid chromatography (HPLC) systems were manufactured by AgilentThe components of the HPLC systems are listed in Table 2.2.

Table 2.2: 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

2.2 Strain Cultivation

2.2.1 Batch Fermentation

The strain Tü2401 was cultivated at a ten-liter [1] 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 CaCO₃. 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 2.3.1.

2.2.2 Media

The used media are listed in Table 2.3

2.3 Sample Preparation

2.3.1 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 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.

2.4 Chromatographic Methods

2.4.1 Thin Layer Chromatography

2.4.2 Ion Exchange Chromatography

2.4.3 Hydrophilic Interaction Chromatography

Hydrophilic Interaction Chromatography (HILIC) was 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 H₂O was used as solvent A, while Acetonitrile comprised solvent B. Detailed method descriptions regarding solvent composition, flow and duration are listed in the appendix.

2.4.4 High Performance Liquid Chromatography

2.4.5 Mass Spectrometry

A test table should be here

Table 2.3: Media components for the cultivation of strain Tü2401. The media were prepared by weighing the specified amounts and solving them in one liter of Milli-Q H₂O. The pH was adjusted with NaOH and HCl.

Name	pH	Component	Amount	Vendor
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	
		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 · 2 H ₂ O	3 g	
		Fe ³⁺ citrate	1 g	
		MnSO ₄ · H ₂ O	200 mg	
		ZnCl ₂	100 mg	
		CuSO ₄ · 5 H ₂ O	25 mg	
		Na ₂ B ₄ O ₇ · 10 H ₂ O	20 mg	
		CoCl ₂ · 6 H ₂ O	4 mg	
		Na ₂ MoO ₄ · 2 H ₂ O	10 mg	

2 Methods

Table 2.4: A test table for HPLC Methods

	Component	Description
HPLC Parameters	System	
	Column	
	Injection volume	50 µL
	Flow	
	Temperature	
	Solvents	Solvent A: H ₂ O Solvent B: Acetonitrile
	Method	Isocratic, 80 % B 60 min
MS Parameters	Capillary Voltage	3500 V
	Temperature	350 °C
	Target Mass	250 m/z

3 Results & Discussion

3.1 Allala

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Figure 3.1: Bioassay with fractions against *E. coli* K12

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Bibliography

- [1] Michelle F Richter et al. “Predictive compound accumulation rules yield a broad-spectrum antibiotic”. In: *Nature* 545.7654 (May 2017), pp. 299–304. ISSN: 0028-0836. URL: <http://dx.doi.org/10.1038/nature22308> http://10.0.4.14/nature22308http://www.nature.com/nature/journal/v545/n7654/abs/nature22308.html%7B%5C%7Dsupplementary-information.

Appendix

3.1 HPLC Methods