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Titel | Title

"Antimicrobial impact of crude metabolite extracts of Streptomyces sp. UV5 and Pseudomonas sp. SPOG282 on the growth of human gut commensals"

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Abstract

The gut microbiome is an important contributor to human health. Changes in the microbiome are linked to intestinal diseases like inflammatory bowel disease, but also extraintestinal diseases, such as cardiovascular disorders. One factor that influence the composition of the gut microbiota are drugs interfering with the growth of gut commensals. Screening for potential side-effect of these compounds on the gut microbiome is an important aspect of precision medicine. Here, I initially adapted a microplate-based cultivation method for screening the impact of metabolites and chemical compounds on the anaerobic growth of representative human gut bacteria. The strain collection consisted of 18 bacterial strains reflecting the phylogenetic and metabolic diversity of the human gut microbiota. After successful cultivation in selected media, growth parameters like growth curves and maximum growth rates, were obtained. In the next step, the effects of known antibiotics were analyzed regarding the growth of selected gut bacteria. Afterwards, bacterial crude methanol extracts generated from the cultures of *Streptomyces* sp. UV5 and *Pseudomonas* sp. SPOG282 were tested on selected strains of the community. Strains UV5 and SPOG282 represent promising candidates for producing novel antibiotics and were previously demonstrated to have antimicrobial activity against aerobically grown gram-negative and gram-positive bacteria. In this study, I show that the inhibitory effect of crude metabolite extracts from these strains extent to selected gut commensals including *Alistipes putredinis, Bifidobacterium longum*, *Collinsella aerofaciens*, *Enterocloster bolteae*, *Escherichia coli* and *Enterococcus faecalis*. Thereby it was shown that the extracts contain antimicrobial compounds with a broader range of action.

I. Introduction

1.1. The importance of the human gut microbiome

The human gut microbiome comprises trillions of microorganisms, including bacteria, fungi and archaea as well as an even higher number of viruses. Most species belong to the six major prokaryotic phyla Actinomycetota, Bacillota, Bacteroidota, Pseudomonadota, Verrucomicrobiota and Euryarcheaota (Bliss& Whiteside 2018). The gut microbiome's significance extends beyond digestion, as it is also involved in various physiological processes and has an important impact on our overall health (Bull&Plummer 2014).

The gut microbiome helps in the breakdown and absorption of nutrients, synthesizes essential vitamins and contributes to the development and function of the immune system. It serves as a critical barrier against pathogenic microbes, playing a protective role by outcompeting harmful invaders and producing antimicrobial compounds (Hou *et al.* 2022). Additionally, the gut microbiome communicates with other body systems, including the nervous system, through complex signaling pathways, and thereby has an influence on the whole metabolism and even the mood (Bull&Plummer 2014; Vyas&Ranganathan 2012).

Recent advances in research have uncovered the gut microbiome's extensive influence on numerous health conditions. Dysbiosis and the imbalance of the gut microbiome have been linked to a variety of disorders, such as inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular and liver diseases (Fan&Pedersen 2021; Boulangé *et al*. 2016; Hrncir 2022). Through the gut-brain axis, which is a bidirectional communication network between the gut and the brain, an imbalanced microbiota can be associated with psychiatric disorders like depression and anxiety (Collins *et al*. 2012; Mayer 2011; Foster&McVey Neufeld 2013).

The gut microbiome's composition and function can be shaped by several factors, including diet, lifestyle, antibiotics as well as other drugs and antimicrobial compounds and also genetics (Cresci&Bawden 2015). With this knowledge, the possibilities of therapeutic interventions for the maintenance or recovery to a healthy microbiome are diverse. The use of probiotics and prebiotics as well as dietary modifications and fecal microbiota transplantation are being explored as potential treatments for various conditions linked to microbiome imbalances (Gagliardi *et al*. 2018). Therefore, it is important to understand the human gut microbiome's complexity and its interactions with the host for the development of personalized medication and targeted therapies (Gagliardi *et al*. 2018; Ho *et al*. 2015).

1.2. Antibiotics and antimicrobial compounds

Antimicrobial compounds are naturally produced by bacteria, fungi and plants and have been exploited as powerful drugs and supplements that have revolutionized medicine and agriculture over the past century. These substances can inhibit the growth or kill pathogenic bacteria and fungi, thereby playing a crucial role in treating infections and preventing the spread of disease (Hutchings *et al*. 2019). The golden area of antibiotic research started with the discovery of penicillin in the early 20th century and successfully reduced mortality rates from bacterial infections (Hariyanto *et al*. 2022; Fleming 1929). Thereby, also the risk of infections during surgery and operation could be significantly reduced (Hutchings *et al*. 2019).

Antibiotics are a subgroup of antimicrobials, which are only effective against bacteria and are often used to treat bacterial infection (Nankervis *et al.* 2016). However, they often also target and kill commensal bacteria from the human gut microbiome, which can lead to dysbiosis and disease (Weersma *et al*. 2020; Blaser 2016).

However, the overuse and misuse of antibiotics in infection treatments and especially agriculture and animal industry has led to significant challenges, most notably the rise of antibiotic-resistant bacteria. As pathogenic bacteria evolve mechanisms to evade antibiotics, treating an increasing number of infectious diseases becomes progressively more difficult. This growing resistance highlights the need for the research and discovery of new antimicrobial compounds (Spellberg *et al*. 2013; Tsamo *et al*. 2021).

1.3. Bacterial secondary metabolite production

Bacterial secondary metabolites are a diverse group of organic compounds produced by bacteria that are not essential for their growth and reproduction (Craney *et al.* 2013). However, they can provide a fitness advantage to the producing organism in various ecological and physiological functions that enhance the bacteria's ability to survive in their environments. Such functions include defense against predators and competitors, facilitation of symbiotic relationships, and communication within microbial communities (Tyc *et al*. 2017).

Antimicrobial compounds as example for bacterial secondary metabolites are produced for the competition against other microorganisms, targeting a variety of cellular processes in microorganisms, such as cell wall synthesis, protein production, DNA replication, and different metabolic pathways (Kapoor *et al*. 2017).

Bacterial secondary metabolites can be classified into several distinct classes based on their chemical structures, biosynthetic pathways, and biological functions (Iqbal *et al*. 2023). Polyketides are characterized by their backbone, which is synthesized by iterative

condensation of simple carboxylic acid building blocks (McDaniel *et al*. 1993). Examples of polyketides include the antibiotics erythromycin and tetracycline as well as the anticancer drug anthracycline (Fang *et al.* 2018; Katz&Baltz 2016).

Non-ribosomal peptides (NRPs) are synthesized by multi-modular enzymes and in contrast to ribosomal peptides without an mRNA template (Bushley& Turgeon 2010). One module can be divided into three different domains: the adenylation (A), thiolation (T) and condensation (C) domain, which catalyze specific reactions in the synthesis of the NRP (Drake *et al*. 2016). These peptides often contain non-standard amino acids as well as hyper-modified amino acids (Walsh *et al*. 2001). Examples of non-ribosomal peptides include the antibiotic vancomycin, the immunosuppressant cyclosporin and the anticancer drug bleomycin (van Wageningen *et al*. 1998; Weber *et al*. 1994; Du *et al*. 2000).

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are produced via the ribosome with an mRNA template as a short precursor peptide, which is later altered by different post-translational modifications (Oman&van der Donk 2010). Examples are the antimicrobial lasso peptide Microcin J25 and the antibiotic nisin (Salomón und Farías 1992; Rogers 1928).

The production of secondary metabolites is triggered by environmental cues like nutrient scarcity or competition from other microbes (Keller 2019). Thereby the production has to be strongly regulated (Jeong *et al*. 2020; Fouillaud&Dufossé 2022). The biosynthetic pathways responsible for producing these compounds are encoded by biosynthetic gene clusters (BGCs), which are believed to be not expressed, i.e. silent, most of the time and only activated during stress conditions (Lee *et al*. 2020). Advances in genomic sequencing and bioinformatics have greatly enhanced the ability to identify and predict the function of these gene clusters, opening new avenues for the discovery of novel secondary metabolites (Adamek *et al*. 2017).

1.4. Biosynthetic gene clusters

The production of antimicrobial compounds, among other secondary metabolites, is encoded by biosynthetic gene clusters (BGCs). These are clusters of genes in the genomes of organisms, particularly bacteria, plants and fungi, encoding all the enzymes and regulatory proteins needed for the production of corresponding natural products (Cimermancic *et al*. 2014; Tyc *et al*. 2017).

A BGC is composed of all of the genes needed for the synthesis of the precursor product, its modification and the transport to the outside of the cell as well as regulatory elements (Martinet *et al*. 2019). This genomic organization ensures the controlled production of the secondary metabolites in response to environmental cues that they are only produced when beneficial for

the producing microorganism (Jeong *et al*. 2020; Fouillaud&Dufossé 2022). A typical example is the BGC responsible for the production of the lipophilic antibiotic thermoactinoamide A found in *Thermoactinomyces vulgaris* (Figure 1).

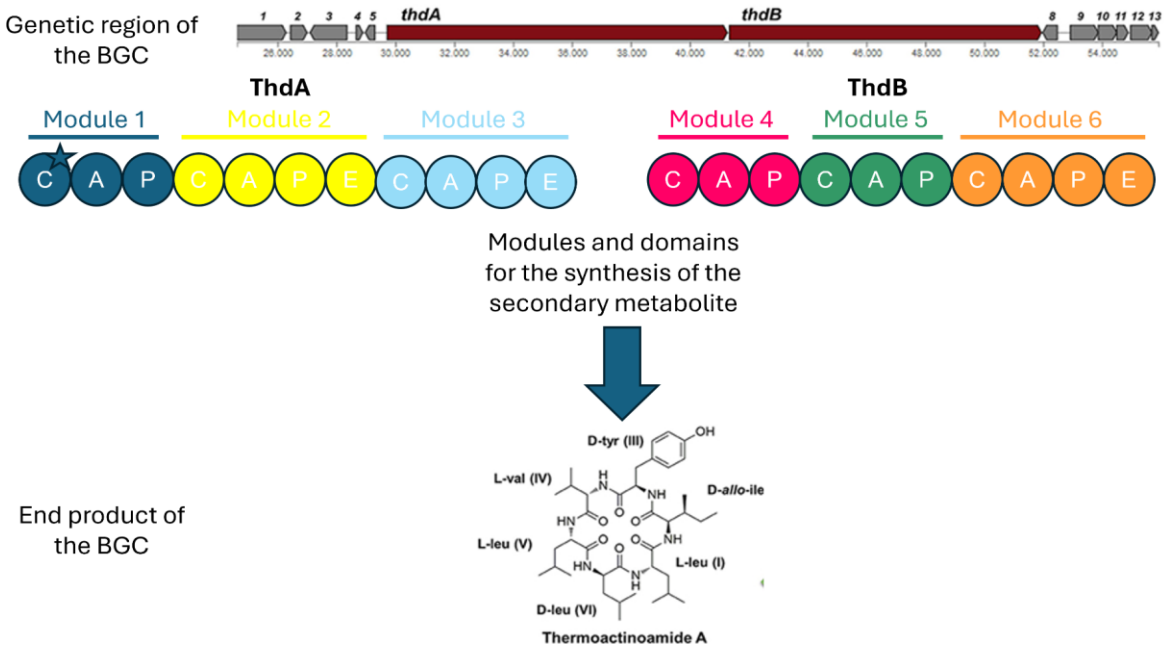


Figure 1: BGC of *Thermoactinomyces vulgaris* is responsible for the production of the antibiotic thermoactinoamide A. This compound is a new cyclic hexapeptide built by a mixture of D- and L- amino acids, along with five minor analogs. The compound has been shown to have a strong inhibitory effect against *Staphylococcus aureus* ATCC 6538. C, condensation domain; A, adenylation; P, peptidyl-carrier protein; E, epimerase; C*, truncated condensation domain, likely inactive. (Figure adapted from Della Sala *et al*. 2020)

Through the advances in whole genome sequencing techniques as well as bioinformatics the identification and analysis of BGCs have accelerated in recent years. This has revealed that many microorganisms comprise several unknown BGCs with the potential production of novel secondary metabolites (Adamek *et al*. 2017). Computational tools and databases, such as antiSMASH (antibiotics and Secondary Metabolite Analysis Shell), NP.searcher, SMURF (Secondary Metabolites Unique Regions Finder), eSNaPD (environmental Surveyor of Natural Product Diversity) and MIBiG (Minimum Information about a Biosynthetic Gene cluster), are used to find BGCs in microbial genomes and link gene clusters with their respective produced metabolite (Blin *et al*. 2023; Li *et al.* 2009; Khaldi *et al.* 2010; Reddy *et al.* 2014; Terlouw *et al*. 2023).

1.5. The production of antibiotics by the genus *Streptomyces*

Streptomyces is a genus of gram-positive, filamentous and often spore-forming bacteria in the phylum Actinomycetota, which are found in various habitats, including terrestrial and marine regions as well as extreme and understudied environments (Khadayat *et al*. 2020; Lee *et al*. 2020). Their linear genomes are characterized by an exceptionally high G + C content of over 70% and the large size of 8-10 Mb (Hopwood 2019).

Streptomyces genomes contain a high number of BGCs for the production of many bioactive secondary metabolites that are used in medicine and agriculture (Ward&Allenby 2018; Nicault *et al*. 2021). The genus is well known for the production of antibiotics and antifungals. Other bioactive agents reported from *Streptomyces* species include antiviral, antitumor, immunosuppressive, antihypertensive, insecticide, plant growth-promoting, herbicidal, antioxidative and cytotoxic agents (Singh und Vaishnav 2022; Nguyen *et al*. 2020; Salwan&Sharma 2020; van Pham et al. 2021; Sharma *et al*. 2021).

The first antibiotic discovered from *Streptomyces* was streptothricin in 1942, followed by well-known antibiotics like tetracycline, munumbicin, and harmaomycin (Castillo *et al*. 2002; Bae *et al*. 2015; Nelson *et al*. 2001). Currently, it is estimated that approximately 70% of known antibiotics derived from microorganisms of the genus *Streptomyces*. However, by an in-depth analysis of their genomes, a large number of so called cryptic BGCs can be found. Here, the produced secondary metabolite is not known or discovered yet. (Chung *et al.* 2021). Therefore, *Streptomyces* are still a valuable target for the discovery of novel antimicrobial compounds (Procópio *et al*. 2012).

1.6. The genus *Pseudomonas* and secondary metabolite production

Pseudomonas, a diverse genus from the phylum Pseudomonadota, encompasses gram-negative, slightly rod-shaped bacteria, known for their motility by one or more polar flagella (Korber *et al.* 2009). They colonize different habitats in the environment including plants, animals and humans, as well as various soils and aquatic ecosystems (Shalev *et al*. 2022; Zhou *et al*. 2021; Boxberger *et a*l. 2021; Butaitė *et al*. 2017; Mulet *et al*. 2020). Species of the genus *Pseudomonas* play an important role in the cycling of different nutrients and thrive as beneficial commensal of plant as well as animal hosts (Guo *et al*. 2015; Zhan *et al*. 2016; Shalev *et al*. 2022). Some species of this genus like *Pseudomonas aeruginosa* are well-known as human pathogens (Diggle&Whiteley 2020). In addition, *Pseudomonas* species are well-known producers of various secondary metabolites with different ecological functions (Saati-Santamaría *et al*. 2022; Rieusset *et al*. 2020).

Pseudomonas species produce a repertoire of different natural products for the survival in extreme conditions as well as for competition against other microbes (Saati-Santamaría *et al*. 2022; Rieusset *et al*. 2020). Some of the best-studied secondary metabolites produced by *Pseudomonas* species are phenazines which can have antibiotic, antitumor, as well as antiparasitic activities (Mavrodi *et al*. 2006; Laursen und Nielsen 2004). Examples of phenazine produced by *Pseudomonas* species are pyocyanin, which is a pathogenicity factor of *Pseudomonas aeruginosa*, and phenazine-1-carboxylic acid, which shows antifungal as well antimicrobial activity against different plant pathogens (Lau *et al*. 2004; Rahme *et al*. 2000; Mavrodi *et al*. 2006).

In addition to phenazines, *Pseudomonas* species are also producers of NRP like pyochelin and pseudomonine, which act as siderophores, especially in the scavenging of iron ions (Budzikiewicz 2004; Anthoni *et al*. 1995). Other examples of secondary metabolites are polyketides and fatty-acid-derived compounds including antimicrobial compounds mupirocin and 2,5-dialkylresorcinols, which exhibit antibacterial as well as antifungal activities (Fuller *et al*. 1971; Nowak-Thompson *et al*. 2003). *Pseudomonas* spp. synthesize also quinolones such as 2-heptyl-3-hydroxy-4-quinolone and other 4-quinolones, which are quorum-sensing molecules for cell-to-cell communication (Miranda *et al*. 2022).

1.7. Aim of this study

Two recent studies investigated the detrimental effect of several antibiotics, but also non-antibiotic drugs on the growth of 38 prevalent and abundant bacterial species of the human gut microbiome (Maier *et al*. 2021 and Maier *et al*. 2018). Additionally, the authors screened for drugs that antagonize the effect of standard antibiotics like erythromycin on abundant *Bacteroides* species but still maintained antimicrobial activity against relevant pathogens (Maier *et al*. 2021).

Using a similar approach, this study aimed to develop a protocol for the semi-high-throughput screening of different antimicrobial compounds on the growth of anaerobic bacterial strains, reflecting the diversity of the healthy human gut microbiota. The validated protocol was subsequently used to evaluate the growth inhibition of known antibiotics, as well as different antimicrobial extracts generated by cultures of *Streptomyces* sp. UV5 and *Pseudomonas* sp. SPOG282 on the growth of a selection of gut bacterial strains.

II. Material and methods

2.1. Bacterial species collection

The bacterial species used in this study were obtained either from the established in-house DOME gut group collection or from DSMZ (German Collection of Microorganisms and Cell Cultures GmbH) (Table 1). The cultures of *Enterococcus faecalis* and *Escherichia coli* were provided by The University Hospital Brno, Czech Republic. Both strains were isolated from stool samples of healthy donors. The isolates without available genome sequences were submitted for whole genome sequencing to the Joint Microbiome Facility of the University of Vienna.

Table 1: Bacterial strains used in the study (^{T}=type strain)

Species name	Strain	Genome accession number
Agathobacter rectalis	DSM 17629	FP929042.1
Akkermansia muciniphila	DSM 22959^T	ABFK00000000.2
Alistipes putredinis	DSM 17216^T	NC_010655.1
Bacteroides caccae	DSM 19024^T	GCA_028539535.1
Bacteroides fragilis	DSM 2151^T	NC_003228.3/CR626927.1
Bacteroides ovatus	DSM 1896^T	FNQD00000000
Bacteroides thetaiotaomicron	DSM 2079^T	CP040530.1
Bacteroides xylanisolvens	DSM 18836^T	FP929033.1
Bifidobacterium longum subs. longum	DSM 20219^T	LR134369.1
Bilophila wadsworthia	LT0012/SQ01	GCA_000185705.2
Collinsella aerofaciens	human fecal isolate	CP024960.1
Escherichia coli	human fecal isolate	CP026755.1
Enterococcus faecalis	human fecal isolate	CP063283.1
Enterocloster bolteae	DSM 15670^T	ABCC00000000.2
Lactobacillus hominis	DSM 23910^T	CAKE01000000
Phocaeicola vulgatus	DSM 1447^T	CP000139
Prevotella copri	DSM 18205^T	ACBX00000000
Roseburia intestinalis	DSM 14610^T	ABYJ00000000

The selected microbial community aimed to represent the core microbiota of the human gut, reflecting the phylogenetic diversity and core metabolic pathways of the gut microbiota in healthy humans.

2.2. Growth conditions

All bacterial strains were cultivated in an anaerobic chamber (2% H_2, 12% CO_2, 86% N_2) and the solutions and media used in the experiment were pre-reduced to become anoxic 48 hours prior to use. The bacteria in this study were either cultivated using modified Gifu Anaerobic Media (mGAM, HiMedia) or Brain Heart Infusion media (BHI, HiMedia) at 37°C (Table 2). Strains ordered from the DSMZ were pre-cultivated in their preferred growth media listed by the DSMZ: Chopped meat medium with carbohydrates (supplemented with vitamin K and hemin) for *A. putredinis* DSM 17216, *Bifidobacterium* medium for *B. longum* subs. longum DSM 20219, PY+X medium for *E. bolteae* DSM 15670 and modified YCFA medium for *R. intestinalis* DSM 14610 (Table 3). Afterward, these strains were either grown using BHI or mGAM media. All media were sterilized by autoclaving at 121°C for 15 minutes.

Table 2: BHI and mGAM media composition in g per liter of medium

BHI broth composition		Modified GAM broth composition	
Beef heart	5 g	Peptone	5 g
Calf brains	12.5 g	Soya peptone	3 g
Disodium hydrogen phosphate	2.5 g	Protease peptone	5 g
D(+)-glucose	2 g	Digestive serum	10 g
Peptone	10 g	Yeast extract	2.5 g
Sodium chloride	5 g	Meat Extract	2.2 g
Final pH (at 25°C)	7.4	Liver Extract	1.2 g
		Dextrose (Glucose)	0.5 g
		Potassium dihydrogen phosphate	2.5
		Sodium chloride	3 g
		Starch soluble	5 g
		L-Cysteine hydrochloride	0.3 g
		Sodium thioglycollate	0.3 g
		L-Arginine	1 g
		Vitamin K1	0.005 g
		Hemin	0.01 g
		L-Tryptophan	0.2 g
		Final pH (at 25°C)	7.3

Table 3: Composition in mg/g or µl/ml per liter of medium used for the pre-cultivation of the ordered DSMZ strains

Chopped meat medium composition		Bifidobacterium medium composition	
Dehydrated cooked meat granules	100 g	Casein peptone, tryptic digest	10 g
Casitone	30 g	Yeast extract	5 g
Yeast extract	5 g	Meat extract	5 g
K_2HPO_4	5 g	Bacto Soytone	5 g
Na-resazurin solution (0.1% w/v)	0.5 ml	Glucose	10 g
D-Glucose	4 g	K_2HPO_4	2 g

Cellobiose	1 g	$MgSO_4$ x 7 H_2O	0.2 g
Maltose	1 g	$MnSO_4$ x H_2O	0.05 g
Starch (soluble)	1 g	Tween 80	1 ml
L-cysteine-hydrochloride	0.5 g	NaCl	5 g
		Cysteine–HCl x H_2O	0.5 g
<u>After autoclaving:</u>			
Hemin solution	10 ml	Salt solution	40 ml
Vitamin K_1 solution	10 ml	Resazurin (25 mg/ 100 ml)	4 ml
Final pH	7.0	Final pH	6.8
<u>Hemin solution:</u> 50 mg Hemin in 1ml 1N NaOH, then filled up to 100 ml with milliQ H_2O and filter sterilized		<u>Salt solution:</u>	
		$CaCl_2$ x 2 H_2O	0.25 g
		$MgSO_4$ x 7 H_2O	0.5 g
		K_2HPO_4	1 g
<u>Vitamin K_1 solution:</u> 0.1 ml Vitamin K_1 in 20 ml 95% ethanol and filter sterilized		KH_2PO_4	1 g
		$NaHCO_3$	10 g
		NaCl	2 g

PY+X medium composition		YCFA medium (modified) composition	
Trypticase peptone (BD BBL)	5 g	Casitone	10 g
Meat peptone (pepsin-digested)	5 g	Yeast extract	2.5 g
Yeast extract	10 g	Glucose	5 g
Salt solution	40 ml	$MgSO_4$ x 7 H_2O	45 mg
Sodium resazurin (0.1% w/v)	0.5 ml	$CaCl_2$ x 2 H_2O	90 mg
L-Cysteine HCl x H_2O	0.5 g	K_2HPO_4	0.45 g
D-Glucose	5 g	KH_2PO_4	0.45 g
		NaCl	0.9 g
<u>Salt solution:</u>		Resazurin	1 mg
$CaCl_2$ x 2 H_2O	0.25 g	$NaHCO_3$	4 g
$MgSO_4$ x 7 H_2O	0.5 g	L-Cysteine HCl	1 g
K_2HPO_4	1 g	Hemin	10 mg
KH_2PO_4	1 g	<u>Volatile fatty acids:</u>	
$NaHCO_3$	10 g	Acetic acid	1.9 ml
NaCl	2 g	Propionic acid	0.7 ml
Final pH	6.8-7.0	iso-Butyric acid	90 µl
		n-Valeric acid	100 µl
		iso-Valeric acid	100 µl
		<u>After autoclaving:</u>	
		Vitamin solution	10 ml
		Final pH	6.7-6.8
		<u>Vitamin solution</u>	
		Biotin	2 mg
		Folic acid	2 mg
		Pyridoxine- HCl x 2 H_2O	10 mg
		Thiamine-HCl x 2 H_2O	5 mg
		Riboflavin	5 mg
		Nicotinic acid	5 mg
		D-Ca-pantothenate	5 mg
		Vitamin B_{12}	0.1 mg
		p-Aminobenzoic acid	5 mg
		Lipoic acid	5 mg

2.3. Identity check of the bacterial strains via 16S rRNA PCR

The identities of the bacterial strains were checked by amplifying the 16S rRNA gene, followed by Sanger sequencing. Bacterial strains were grown on agar plates between 2-6 days, depending on the strain and media used. Bacterial biomass (1 µl loop) from the BHI or mGAM agar plates was resuspended in 200 µl MQ water. DNA was extracted using Chelex-100 Resin (Biorad) using the following protocol: First, the suspension was centrifuged for 2 min at 15500 rcf and the supernatant was discarded. The pellet was dissolved in 100 µl 5% Chelex-water solution. After boiling in a thermoblock at 100°C for 10 min, the suspension was centrifuged again using the same settings as listed above. The supernatant with the extracted DNA was transferred to a new Eppendorf tube. The concentration of the extracted DNA was measured using the Nanodrop and diluted to 10 ng/µl.

16S rRNA gene PCR. The 16S rRNA gene of the strains was amplified by PCR using the primer pair 616v/1492r (Juretschko *et al.* 1998; Kane *et al.* 1993). Each 25 µl PCR reaction contained the reagents listed in Table 4, and 2µl of template was used. Cycling was done in a Biorad T100 thermocycler with 35 cycles (Table 4).

Table 4: PCR reagents and PCR program used for 16S rRNA gene amplification

PCR reagents	amount	final - conc.
MilliQ water	16.8 µl	
10X Dream Taq buffer (Thermo Scientific)	2.5 µl	1X
dNTP mix (2 mM) (Thermo Scientific)	2.5 µl	0.2 mM
F-primer 616v (5 µM)	1 µl	0.2 µM
R-primer 1492r (5 µM)	1 µl	0.2 µM
BSA (20 µg/µl) (Thermo Fisher)	0.1 µl	0.08 mg ml^{-1}
Dream Taq Polymerase (5 U/µl) (Thermo Fisher)	0.1 µl	0.02 U

PCR step	Temperature	Time
Initial Denaturation	95°C	3 min
Denaturation	95°C	30 sec
Annealing	52°C	30 sec
Elongation	72°C	60 sec
Final elongation	72°C	5 min
Hold	4°C	infinite

Agarose gel electrophoresis: An agarose gel electrophoresis was performed using a 1% agarose gel. Initially, 0.8 g of LE (low electroendosmosis) agarose (Biozym Scientific GmBH) was dissolved in 80 ml of TBE (TRIS-Borat-EDTA) buffer and heated in a microwave until dissolved. The liquid gel was stained using 0.1X GelRed (Biotium Inc.), transferred into gel trays and solidified. The gel was loaded with 2 µl of the samples mixed with 1.5 µl 6x ready-to-use loading dye (Thermo Fisher). As a DNA ladder, 4 µl of GeneRuler 1 kb Plus DNA Ladder (Invitrogen) was used. The gel was run at 100 V for 30 minutes and checked using the Bio-Rad gel image system.

Purification of the PCR product. The PCR product was purified for sequencing using the ExoSAP-IT enzyme (Thermo Fisher). In a PCR tube, 15 µl of the PCR product and 6 µl of the ExoSAP enzyme were combined. The tubes were incubated in a thermal cycler for 4 minutes at 37°C followed by 1 minute at 80°C and held at 12°C.

Sanger sequencing and sequence analysis: The purified samples were submitted for Sanger sequencing at the Microsynth company (https://srvweb.microsynth.ch/). The quality of the sequences was checked by the purity of the nucleotide chromatogram. The obtained sequences were analyzed by EzBioCloud's identification service (https://www.ezbiocloud.net/identify) to prove the classification of the strains before further use.

2.4. Glycerol stock preparation for long-term storage

After confirmation of the strain identities, glycerol stocks were prepared for long-term storage at -75°C. At first, the strains were cultured in their preferred liquid growth media (BHI or mGAM) for 2-6 days depending on the bacterial species, then 1 ml of the culture was added to 1 ml of a 40% glycerol solution (final concentration of glycerol stock: 20%, for *B. wadsworthia* 5% DMSO was added)). Five replicates for each strain were prepared and the stocks were immediately frozen in liquid N_2 and stored at -75°C.

2.5. Generation of growth curves

The glycerol stocks of the bacterial isolates were inoculated in their preferred liquid media (BHI or mGAM) followed by anaerobically growth at 37°C overnight. The overnight cultures were diluted 1:100 in fresh liquid media for a second consecutive overnight incubation. The absorbance at 600 nm (OD_{600}) of the cultures obtained after the second overnight cultivation was measured and the cultures were subsequently diluted to a starting OD_{600} of 0.05. Cultures for measuring growth curves were then started in 96 well-plates.

Initially, the growth curves were acquired by measuring the OD_{600} every 2 hours. For that the 96-well plates were set up in a 96-well plate reader inside an anaerobic tent. OD measurements were always taken after 1 min of linear shaking of the 96-well plate. Depending on the growth of the different strains, incubation times ranged from 24 to 48 hours (Supplementary Table S1). Based on the first results, the protocol was adapted for subsequent measurements, by measuring the OD_{600} every 5 minutes with 25 seconds of linear shaking. For the first measurement, four technical replicates were used per strain, this was changed in later runs to 5 or 10 replicates to increase statistical power. Additionally, in wells between different species, blank measurements were implemented where only the absorbance of the growth media was measured over time (seen as background absorbance).

The average of the blank measurements was taken over time and subsequently subtracted from the OD_{600} data obtained from the bacterial cultures. The average OD_{600} of the different technical replicates of the same strain was calculated as well as the standard deviation between the replicates. The corrected average OD_{600} data of the bacterial strain was then plotted over the incubation time in Excel to generate growth curves for all tested strains. The standard deviation between the replicates was indicated as error bars in the graph.

2.6. Bioinformatic analysis of growth parameters

For further characterization of the growth of the bacterial strains growth parameters were obtained: maximum growth rate, doubling time and area under the curve (AUC). For calculating these parameters two bioinformatic tools were used, the R package 'growthcurver' (Sprouffske and Wagner, 2016) and the Python package anaconda (Swain *et al.*, 2016). The results of the two different methods were compared to see if the two tools provided similar outcomes.

2.6.1. R package 'growthcurver'

A tab-delimited file (.txt) was created with the absorbance data obtained from the growth experiments, containing the time points of each measurement, the average of the blank measurement replicates and the measurements of each sample at each time point (example - Supplementary Figure S2).

Blank measurements where the absorbance significantly increased over time were removed from the analysis due to potential contamination. Also, wells containing absorbance measurements of bacterial samples that significantly differed from the average of the other replicates of the same strain were removed from further analysis. This could be measurements with spikes, uneven growth or data with high starting OD_{600}.

Additionally, the absorbance data was filtered to contain only measurements taken during the exponential growth phase. For this, the OD_{600} measurements were plotted logarithmically over

time, an exponential trendline was created for the values and all points not following this distribution were removed as they already reached the stationary phase.

The data was then loaded into R using the following command:

Input_data = read.table("/input_file.txt", header=T, sep="\t", stringsAsFactors=F)

Next, the background absorbance was subtracted from the actual absorbance data of the bacterial sample and a logistic function was fitted to the data. From this function, the growth was analyzed and an output table was created containing the calculated growth parameters for all of the replicates for the bacterial sample:

Output_data = SummarizeGrowthByPlate(input_data, bg_correct="blank")

The different growth parameters include maximum growth rate, doubling time and AUC. The specific data was extracted from the table using the following code:

Output_data$r #maximum growth rate

Output_data$t_gen #doubling time

Output_data$auc_e #AUC

The output data table was saved in a tab-separated file for importing into Excel:

write.table(Output_data, file = output_file_name, quote = FALSE, sep = "\t", row.names = FALSE)

The reliability of the logistic function fitted to the input data was checked:

library("dplyr")

output_data %>% filter(note != "")

2.6.2. Bioinformatic analysis – Python package ‘Anaconda’

The input data was prepared the same way as it was done for the R package ‘growthcurver’. The file fitderivgui.py was debugged and run in Python: Anaconda works with a graphical user interface where the corrected and filtered input data was uploaded.

After the program was run on the input data, the results were displayed in two graphs (example in Figure 2): The first showed the calculated growth curve of the bacterial strain ($logOD_{600}$ over time) and the second plot showed the changes in the inferred growth rate over time. The numerical results of the fitting procedure included different growth estimates like the average maximum growth rate, the doubling time, the maximum OD_{600} reached and the lag time of all

the replicates. Additionally, the standard deviation between the replicates was depicted. Also, the maximum likelihood (log) of the fit to the data was calculated.

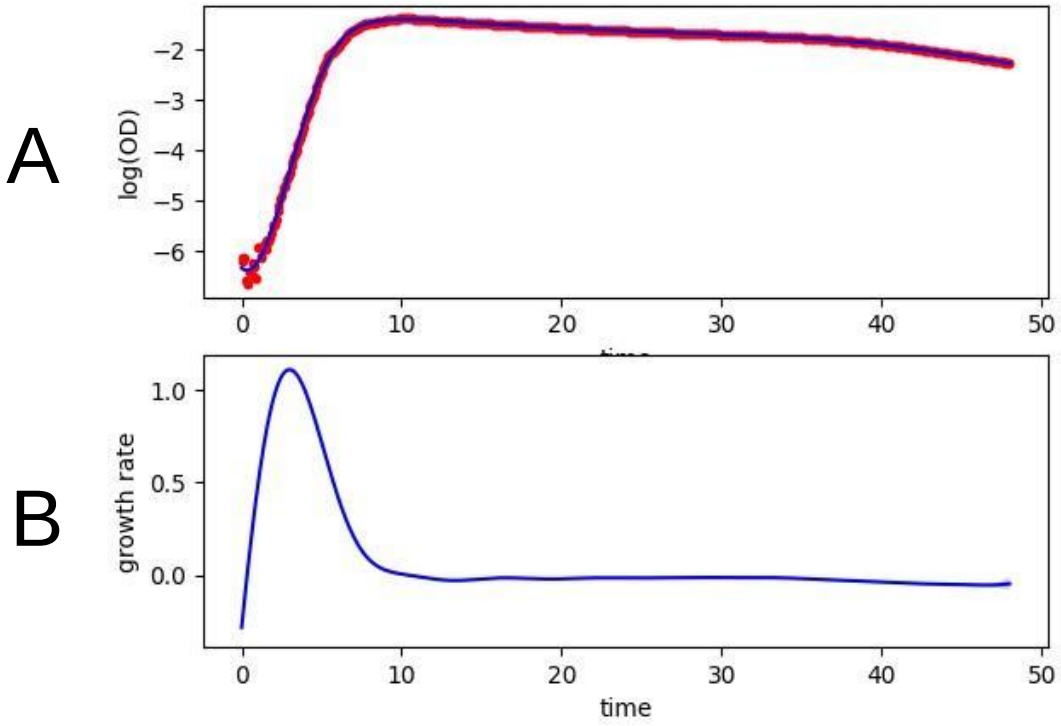


Figure 2: One example of graphs generated with the Python package Anaconda. A) the growth curve of the strain ($logOD_{600}$ over time). B) change in the growth rate over time.

Error corrections were done as follows, if the data showed a poor fit, the upper bound of the hyperparameters was decreased. If the inferred growth rate was too noisy, either the lower bound of the hyperparameters was increased (to prevent over-fitting) or the upper bound on the hyperparameters was decreased (for flexibility).

2.7. Analyzing the growth pattern in the 96-well plate

In order to analyze the reliability of the measurements in the plate reader, single bacterial strains (*B. longum* subs. longum DSM 20219 and *E. coli* - human fecal isolate) were grown in all wells of a 96-well plate (except for the first and last column of the plate as it was known from previous experiments that the growth in these wells is slower due to a higher evaporation of the bacterial cultures). The absorbance was measured for 24 hours. Growth curves and growth parameters were then calculated. The bacterial growth data from all wells were analyzed to assess whether the plate position had a significant effect on the growth of the strains.

2.8. Testing the impact of known antibiotics on the growth of the bacterial strains

At first, the antibiotic ampicillin (Sigma Aldrich) was prepared with a final concentration of 100 µM: For this 0.07 g of ampicillin was dissolved in 2 ml milliQ H_2O and filter-sterilized (pore size 0.45 µm). Twenty µl of the antibiotic was added to 80 µl of the bacterial culture in the 96-well plate to reach a final concentration of ampicillin of 20 µM. The same was done for the antibiotic gentamicin (here 0.1 g gentamicin (Carl-Roth) was dissolved in 2 ml milliQ H_2O). In the plate reader, the OD_{600} of the bacterial samples was measured over time with 5 or 10 replicates. As control, the OD_{600} of the bacterial sample using 10 replicates without the addition of ampicillin or gentamicin was measured. In addition, growth parameters, especially the maximum growth rate, for both treatments were calculated. By comparing the growth curves as well as the calculated maximum growth rate the effect of the antibiotic on the growth of the bacterial strains was evaluated.

2.9. Testing the impact of bacterial metabolite extract on the growth of the bacterial strains.

The first bacterial extract tested, derived from *Streptomyces* sp. nov. UV5, isolated from Antarctic soils (grown in ISP2 medium at 21°C, harvested after 5 days). Extracts from this strain were shown to inhibit the aerobic growth of gram-positive (e.g. *Micrococcus luteus*) and gram-negative (e.g. *Escherichia coli*) bacteria, including multi-drug resistant bacteria (Kralova, unpublished). As a potential gram-negative growth inhibitor, a novel anthracycline was identified, while the potential gram-positive growth inhibitor has yet to be identified. However, the newest findings indicated that the anthracycline could also be the antimicrobial effector against gram-positive bacteria (Králová, unpublished). The metabolite profile of the UV5 extract can be seen in Figure 4.

The second bacterial extract used was generated from *Pseudomonas* sp. nov. SPOG282, isolated from the Schlöppnerbrunnen peat, Germany (grown in KingsB medium at room temperature, harvested after 5 days). It was shown that extracts of this strain inhibit the aerobic growth of gram-positive bacteria (e.g. *M. luteus)* as well as gram-negative bacteria, e.g. *Pseudomonas putida* (Flieder, unpublished). A potential novel lasso peptide was identified by mass spectrometry analysis (annotated metabolite profile in Figure 3) as the most likely compound responsible for the growth inhibition (Flieder, unpublished).

Cultures for screening were grown as before. The UV5 extract was initially added to the cultures in smaller volumes, e.g. 1, 2 and 5 µl. In order to increase the inhibitory effect, higher volumes of the extract were used later (5, 10 and 20 µl). The SPOG282 extract was added to the cultures with a volume of 20 µl. As a control, cultures without the extract were grown in the

same 96-well plate. The absorbance data was again used for the calculation of growth curves and further analysis of growth parameters. The acquired growth curves and parameters were compared between the extract treatment and the controls, thereby the effect of the extracts on the growth of the bacterial strains could be evaluated. In additional trials, a modified UV5 extract where the production of the antimicrobial natural products is upregulated, was tested in different volumes on the growth of the gut commensals. A potentially higher antimicrobial activity of the extract was shown previously in disc-diffusion assays,

As the bacterial extract was dissolved in methanol, also the effect of methanol on the growth of the bacterial strain was evaluated to ensure that the inhibitory effect came from the antimicrobial compounds in the extract and not from the solvent. This was done by adding 20 µl of methanol to the cultures and measuring the growth as described above.

The growth was inhibited when the extract led to a significant decrease in the growth curve as well as a significant reduction in the calculated maximum growth rate. A student´s T-test was done to show statistical significance.

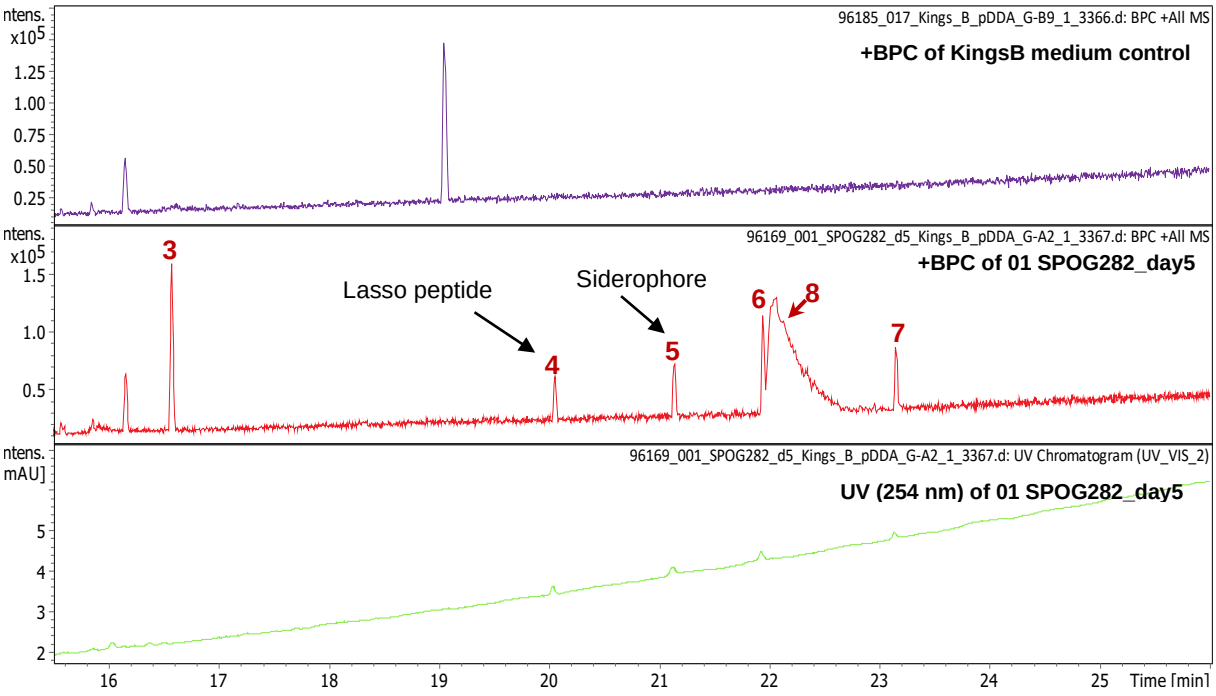


Figure 3: Annotated metabolite profile of the *Pseudomonas* sp. SPOG282 extract (grown in KingsB medium at room temperature for 5 days): Peak 3) modified linear peptide, likely new NP. 4) new lassopeptide. 5) Non-identified siderophore. 6) & 7) congener of 5). 8) Non-identified.

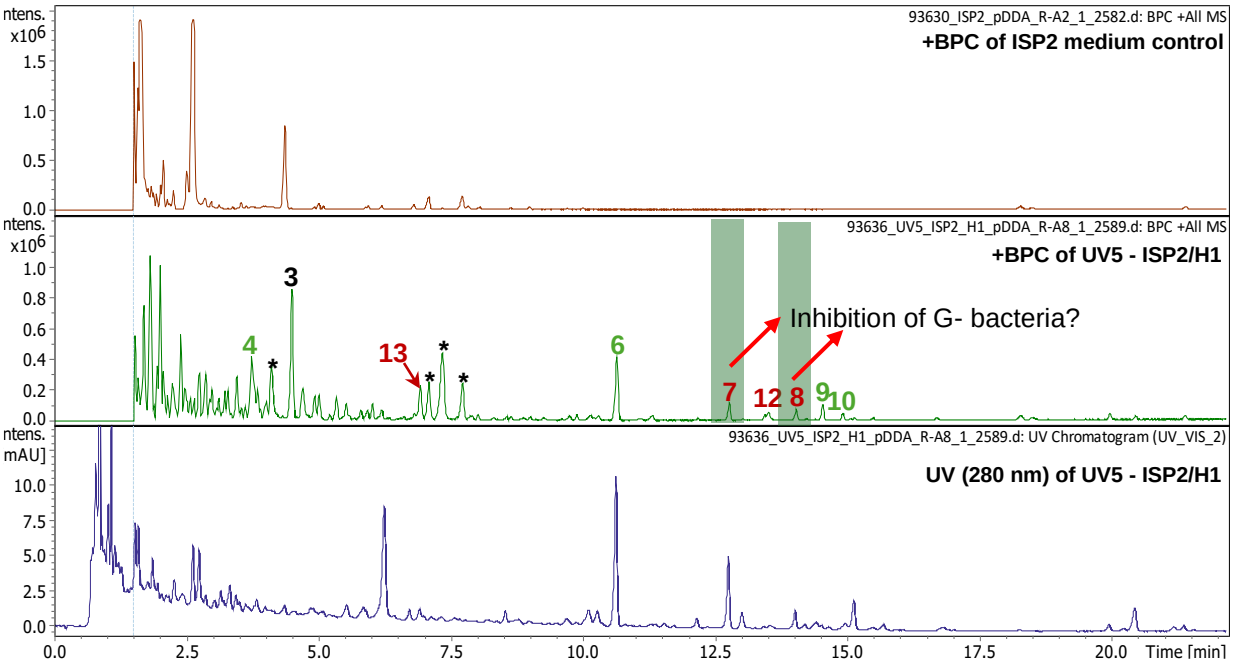


Figure 4: Annotated metabolite profile of the *Streptomyces* sp. UV5 extract (grown in ISP2 medium at 21°C for 5 days): Peak 3) γ-L-glutamyl-L-leucine. 4) Salinichelin C. 5) Salinichelin E. 6) Saccharothrixmicine A or isomer (Atramycin A). 7) Non-identified anthracycline. 8) Non-identified anthracycline. 9) Tambromycin A (or isomer). 10) Tambromycin C (or isomer). 12) New Tambromycin congener. 13) Non-identified.

III. Results

3.1. The gut strain collection was correctly identified by Sanger sequencing

Screening of the 16S rRNA gene sequences revealed the correct identity of all cultivated bacterial strains and enabled their use in subsequent workflow (Table 5). Growth curves, as well as growth parameters, were obtained for all strains. A more in-depth analysis of the effect of different bacterial extracts was done for eight bacterial strains: *A. putredinis* DSM 17216, *B. fragilis* DSM 2151, *B. longum* subs. longum DSM 20219, *C. aerofaciens* (human fecal isolate), *E. boltae* DSM 15670, *E. coli* (human fecal isolate), *E. faecalis* (human fecal isolate), and *L. hominis* DSM 23910.

Table 5: 16S rRNA gene amplicon sequencing results of the grown bacterial strains confirmed their identity (^{T}=type strain).

Collection identity	16S rRNA gene sequencing results (top hit strain)	Sequence similarity to top hit strain
Agathobacter rectalis DSM 17629	*Agathobacter rectalis* DSM 3377	99.5 %
Akkermansia muciniphila DSM 22959^T	*Akkermansia muciniphila* DSM 3377	100 %
Alistipes putredinis DSM 17216^T	*Alistipes putredinis* DSM 17216	100 %
Bacteroides caccae DSM 19024^T	*Bacteroides caccae* DSM 19024	99.1 %
Bacteroides fragilis DSM 2151^T	*Bacteroides fragilis* DSM 2151	99.4 %
Bacteroides ovatus DSM 1896^T	*Bacteroides ovatus* DSM 1896	99.7 %
Bacteroides thetaiotaomicron DSM 2079^T	*Bacteroides thetaiotaomicron* DSM 2079	99.6 %
Bacteroides xylanisolvens DSM 18836^T	*Bacteroides xylanisolvens* XB1A	99.7 %
Bifidobacterium longum subs. longum DSM 20219^T	*Bifidobacterium longum* subs. longum DSM 20219	100 %
Bilophila wadsworthia LT0012/SQ01	*Bilophila wadsworthia* ATCC 49260	99.1 %
Collinsella aerofaciens human fecal isolate	*Collinsella aerofaciens* CPO 24160-S	99.6 %
Escherichia coli isolate from human feces	*Escherichia coli* DSM 30083	98.9 %
Enterococcus faecalis isolate from the hospital	*Enterococcus faecalis* DSM 20478	99.3 %
Enterocloster bolteae DSM 15670^T	*Enterocloster bolteae* DSM 15670	99.6 %
Lactobacillus hominis DSM 23910^T	*Lactobacillus hominis* DSM 23910	99.9 %

Phocaeicola vulgatus DSM 1447^T	*Phocaeicola vulgatus* DSM 1447	99.7 %
Prevotella copri DSM 18205^T	*Prevotella copri* DSM 18205	99.8 %
Roseburia intestinalis DSM 22959^T	*Roseburia intestinalis* DSM 14610	99.8 %

3.2. Successful growth of the gut commensals in the 96-well plate

Depending on the medium preference of each species, either mGAM or BHI was used for growth tests and OD_{600} measurements (Table 6). The growth curves were plotted over time versus the absorbance at 600 nm. The obtained growth curves of the bacterial species can be seen in Figure 5. The growth curves of all of the strains showed the typical bacterial growth with a short lag-phase (not for all species), an exponential growth or log-phase followed by a stationary phase. The time needed to reach stationary phase differed between the species and ranged from 3 hours for *E. faecalis* (human fecal isolate) to 14 hours in *B. fragilis* DSM 2151. *E. bolteae* DSM 15670 showed a biphasic growth with two log-phases with a short stationary phase in between. Replicates of the bacterial sample in the first and last column of the plate showed an increased reduction in absorbance compared to the other replicates (e.g. *E.coli* fecal isolate: OD_{600} of the replicates in the first and last column was 0.05 after 48 hours, for all other replicates 0.15). This occurred due to a faster evaporation in those columns. Therefore, the first and last column from the 96-well plate was excluded from the analysis.

Table 6: Bacterial species and the preferred growth media (^{T}=type strain).

Bacterial strain	Preferred growth media
A. muciniphila DSM 22959^T	BHI
A. putredinis DSM 17216^T	BHI
A. rectalis DSM 17629	BHI/mGAM
B. caccae DSM 19024^T	BHI/mGAM
B. fragilis DSM 2151^T	BHI/mGAM
B. longum subs. longum DSM 20219^T	BHI
B. ovatus DSM 1896^T	BHI
B. thetaiotaomicron DSM 2079^T	BHI/mGAM
B. wadsworthia LT0012/SQ01	BHI
B. xylanisolvens DSM 18836^T	BHI/mGAM
C. aerofaciens human fecal isolate	BHI
E. bolteae DSM 15670^T	BHI/mGAM
E. coli isolate from human feces	BHI/mGAM
E. faecalis isolate from the hospital	BHI
L. hominis DSM 23910^T	BHI
P. copri DSM 18205^T	mGAM
P. vulgatus DSM 1447^T	BHI
R. intestinalis DSM 22959^T	mGAM

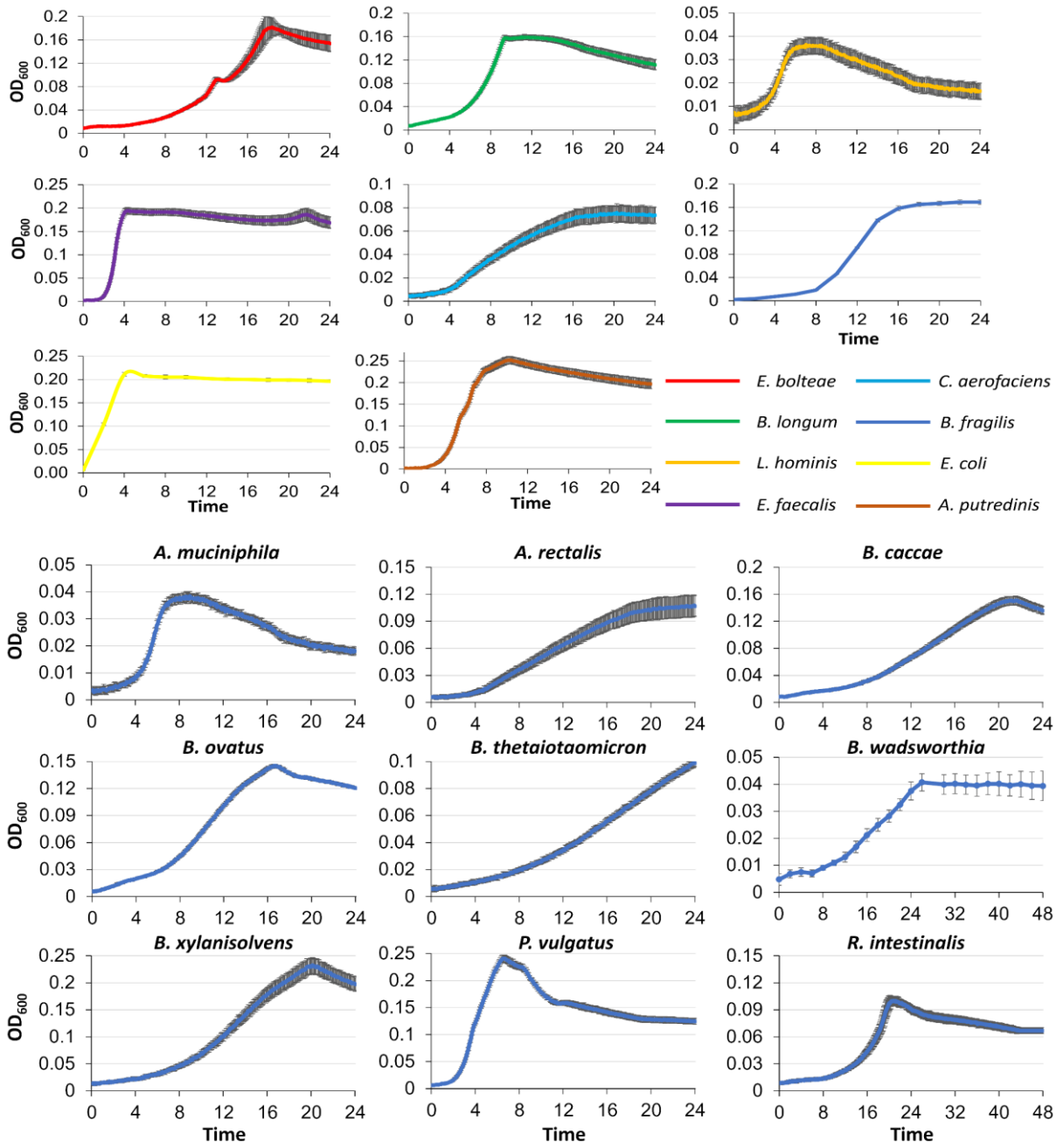


Figure 5: **Growth curves of the bacterial strains.** The growth curves were generated by the measurement of OD_{600} every five minutes. For *B. fragilis* DSM 2151, *B. wadsworthia* LT0012/SQ01 and *E. coli* (human fecal isolate), OD_{600} measurements were done every 2 hours. Background absorbance (media alone) was subtracted from the sample absorbance. Replicates from the first and last column of the 96-well plate were excluded. The average of the replicates (n = 10) was calculated and depicted as one growth curve. The error bar indicates the standard deviation between the replicates.

3.3. Bioinformatic tools Growthcurver and Anaconda did not differ significantly in the calculation of the maximum growth rate

There was no significant difference between the growth rate results of the two bioinformatic tools 'Growthcurver' and 'Anaconda' (Table 7), therefore for later calculations only 'Growthcurver' was used (Students T-test summary: p= 0.465364539514325). The maximum growth rate varied between the species from a minimum of 0.16 in *B. wadsworthia* LT0012/SQ01 to a maximum of 2.2 doublings per hour in *E. faecalis* (human fecal isolate, Table 7). Accordingly, the doubling times ranged from 19 minutes in *E. faecalis* (human fecal isolate) to more than 4 hours in *B. wadsworthia* DSM LT0012/SQ01 (Table 7). The AUC was between 1.82 for *P. copri* DSM 18205 to 481.45 for *A. putredinis* DSM 17216 (Table 7).

Table 7: Comparison of maximum growth rate between the R package 'Growthcurver' and Python package Anaconda as well as doubling time and area under the curve for all of the strains (^{T}=type strain).

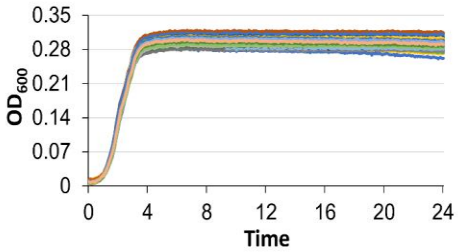
	Maximum growth rate (h^{-1}) - 'Growthcurver')	Maximum growth rate (h^{-1}) - 'Anaconda'	Doubling time (in h)	Area under the curve (AUC)
A. muciniphila DSM 22959^T	0.786	0.672	0.90	33.55
A. putredinis DSM 17216^T	1.077	1.108	0.65	481.45
A. rectalis DSM 17629	0.302	0.373	2.32	86.35
B. caccae DSM 19024^T	0.223	0.328	3.11	106.81
B. fragilis DSM 2151^T	0.455	0.429	1.56	166.41
B. longum subs. longum DSM 20219^T	0.456	0.460	1.53	308.15
B. ovatus DSM 1896^T	0.348	0.450	1.99	120.62
B. thetaiotaomicron DSM 2079^T	0.192	0.179	3.61	60.15
B. wadsworthia LT0012/SQ01	0.163	0.100	4.36	17.14
B. xylanisolvens DSM 18836^T	0.290	0.207	2.47	136.98
C. aerofaciens human fecal isolate	0.692	0.446	1.03	72.55
E. bolteae DSM 15670^T	0.202	0.381	3.57	149.92
E. coli isolate human fecal isolate	1.856	1.784	0.37	397.70
E: faecalis human fecal isolate	2.227	2.245	0.31	270.03

L. hominis DSM 23910^T	0.684	0.526	1.07	33.12
P. copri DMS 18205^T	0.255	0.198	0.99	1.82
P. vulgatus DSM 1447^T	0.190	0.159	3.67	41.96
R. intestinalis DSM 14610^T	0.188	0.234	3.74	140.09

3.4. The position in the 96-well plate had no substantial effect on the growth of the bacterial replicate

To screen for any significant effects of the positions of the wells on the growth of the bacteria on the 96-well plates, *E. coli* (human fecal isolate) and *B. longum* subs. longum DSM 20219 were grown in all wells, except the first and last column. For *E. coli* (human fecal isolate) no significant effect on the growth was observed. For all of the replicates, the growth curves were similar and also a comparable maximum OD_{600} as well as maximum growth rate was observed. The maximum OD_{600} ranged from 0.28 to 0.32 and the maximum growth from 1.7 to 2.4 (Figure 6). For *B. longum* subs. longum DSM 20219 similar results were observed, the maximum OD_{600} (except three significant outliers) ranged between 0.14 and 0.17 and the maximum growth rate between 0.39 and 0.53 (Figure 7).

A



B Maximum OD_{600}

	1	2	3	4	5	6	7	8	9	10	11	12
A	xxx	0,283	0,294	0,291	0,289	0,294	0,292	0,298	0,303	0,305	0,295	xxx
B	xxx	0,284	0,289	0,305	0,303	0,296	0,299	0,310	0,304	0,311	0,310	xxx
C	xxx	0,282	0,301	0,308	0,292	0,294	0,295	0,293	0,297	0,301	0,318	xxx
D	xxx	0,287	0,308	0,304	0,312	0,318	0,313	0,309	0,312	0,312	0,304	xxx
E	xxx	0,287	0,298	0,309	0,320	0,299	0,303	0,292	0,299	0,291	0,310	xxx
F	xxx	0,312	0,312	0,304	0,306	0,315	0,296	0,306	0,306	0,302	0,296	xxx
G	xxx	0,297	0,297	0,305	0,305	0,310	0,301	0,298	0,302	0,291	0,308	xxx
H	xxx	0,297	0,292	0,304	0,301	0,306	0,308	0,307	0,298	0,304	0,301	xxx

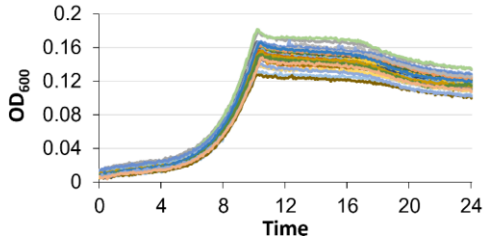
C Maximum growth rate

	1	2	3	4	5	6	7	8	9	10	11	12
A	xxx	2,130	2,127	2,120	2,002	2,021	1,747	2,046	1,936	1,849	1,868	xxx
B	xxx	2,188	2,072	1,984	1,894	2,070	1,692	1,936	1,871	2,265	1,939	xxx
C	xxx	2,384	2,280	2,192	2,194	2,308	2,267	2,193	2,113	2,243	2,186	xxx
D	xxx	2,242	2,222	2,238	2,205	2,262	2,235	2,197	2,280	2,199	2,233	xxx
E	xxx	2,352	2,217	2,205	2,228	2,231	2,263	2,394	2,355	2,415	2,049	xxx
F	xxx	2,004	2,123	2,242	2,090	2,215	2,242	2,261	2,276	2,251	2,325	xxx
G	xxx	2,154	2,172	2,030	2,105	2,262	2,198	2,147	2,301	2,149	2,271	xxx
H	xxx	2,047	2,176	2,046	1,894	2,087	2,142	2,121	2,142	2,090	2,184	xxx

Figure 6: Growth parameters of *E. coli* (human feacal isolate). The first and last column were excluded on the basis of a decreased growth observed in these wells in the previous experiments. A) Growth curves in 80 wells of the 96-well plate. B) Heat map of the maximum

OD_{600}, dark blue indicates lowest values and dark red highest values. C) Heat map of maximum growth rate of the replicates, dark blue indicates lowest values and dark red highest values.

A



B Maximum OD_{600}

	1	2	3	4	5	6	7	8	9	10	11	12
A	xxx	0.152	0.148	0.178	0.194	0.160	0.150	0.153	0.159	0.167	0.146	xxx
B	xxx	0.115	0.153	0.156	0.152	0.165	0.151	0.153	0.158	0.151	0.154	xxx
C	xxx	0.153	0.153	0.153	0.160	0.169	0.157	0.140	0.140	0.132	0.182	xxx
D	xxx	0.156	0.149	0.157	0.128	0.204	0.158	0.156	0.156	0.157	0.156	xxx
E	xxx	0.159	0.158	0.156	0.154	0.159	0.161	0.160	0.163	0.167	0.158	xxx
F	xxx	0.155	0.153	0.157	0.159	0.154	0.158	0.155	0.158	0.161	0.164	xxx
G	xxx	0.155	0.158	0.158	0.156	0.156	0.157	0.167	0.163	0.165	0.157	xxx
H	xxx	0.158	0.152	0.152	0.156	0.154	0.156	0.166	0.152	0.140	0.148	xxx

C Maximum growth rate

	1	2	3	4	5	6	7	8	9	10	11	12
A	xxx	0.399	0.428	0.412	0.431	0.432	0.441	0.404	0.470	0.480	0.458	xxx
B	xxx	0.461	0.452	0.449	0.451	0.435	0.453	0.458	0.410	0.451	0.437	xxx
C	xxx	0.445	0.445	0.491	0.498	0.391	0.481	0.459	0.499	0.534	0.423	xxx
D	xxx	0.437	0.484	0.482	0.532	0.434	0.489	0.503	0.511	0.504	0.504	xxx
E	xxx	0.448	0.486	0.469	0.485	0.481	0.483	0.453	0.448	0.438	0.452	xxx
F	xxx	0.430	0.461	0.486	0.473	0.471	0.466	0.480	0.456	0.476	0.448	xxx
G	xxx	0.413	0.482	0.444	0.479	0.476	0.455	0.391	0.477	0.434	0.468	xxx
H	xxx	0.459	0.451	0.484	0.446	0.485	0.445	0.467	0.492	0.430	0.473	xxx

Figure 7: Growth parameters of *B. longum* subs. longum DSM 20219. The first and last column were excluded on the basis of a decreased growth observed in these wells in the previous experiment. A) Growth curves in 80 wells of the 96-well plate. B) Heat map of the maximum OD_{600}, dark blue indicates lowest values and dark red highest values. C) Heat map of maximum growth rate of the replicates, dark blue indicates lowest values and dark red highest values.

3.5. The antibiotics ampicillin and gentamicin inhibited the growth of some but not all bacterial strains

A concentration of 20 µM ampicillin was sufficient for full growth inhibition of the tested isolates including gram-positive and gram-negative bacteria (*B. longum* subs. longum DSM 20219, *E. faecalis* (human fecal isolate) and *L. hominis* DSM 23910). This was observed by the comparison of the growth curves as well as the calculated maximum growth rate between the control and treatment group, where the growth rate decreased to 0 in the treatment group (Figure 8). In contrast, the same concentration led to no significant inhibition of the growth of *E. bolteae* DSM 15670 (Figure 9).

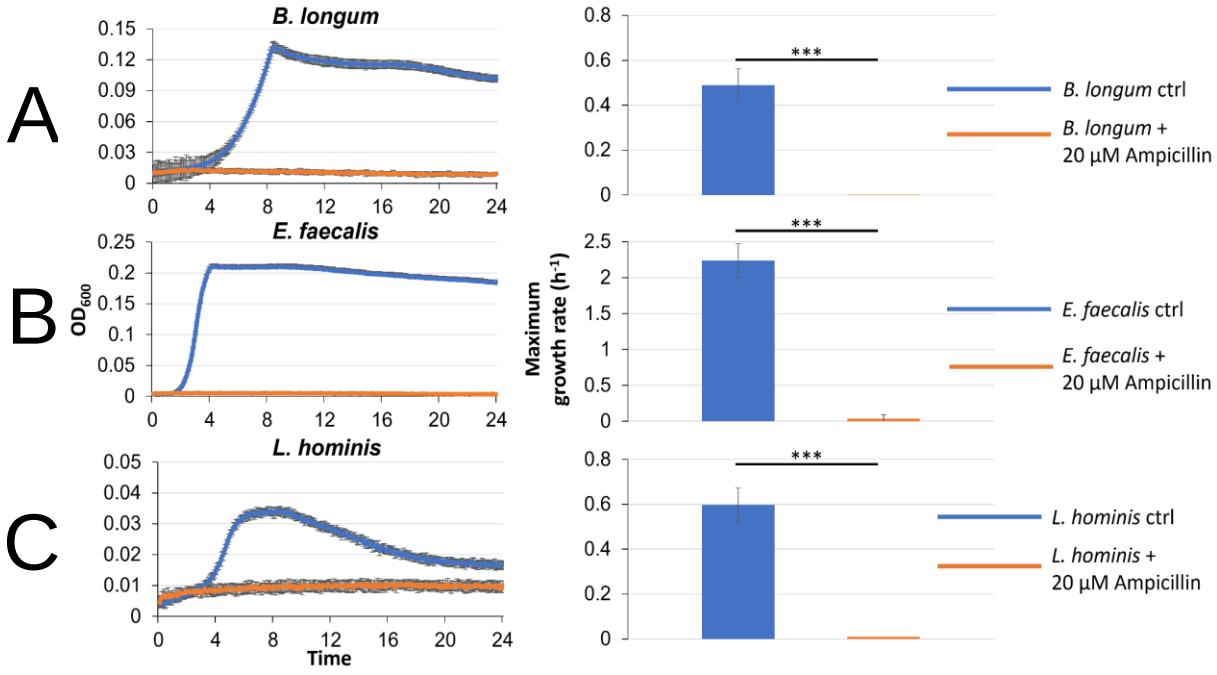


Figure 8: Growth curves and calculated maximum growth rate of three bacterial strains with and without the addition of 20 µM Ampicillin: A) *B. longum* subs. longum DSM 20219. B) *E. faecalis* (human fecal isolate). C) *L. hominis* DSM 23910. Significance: ***= $p < 0.005$

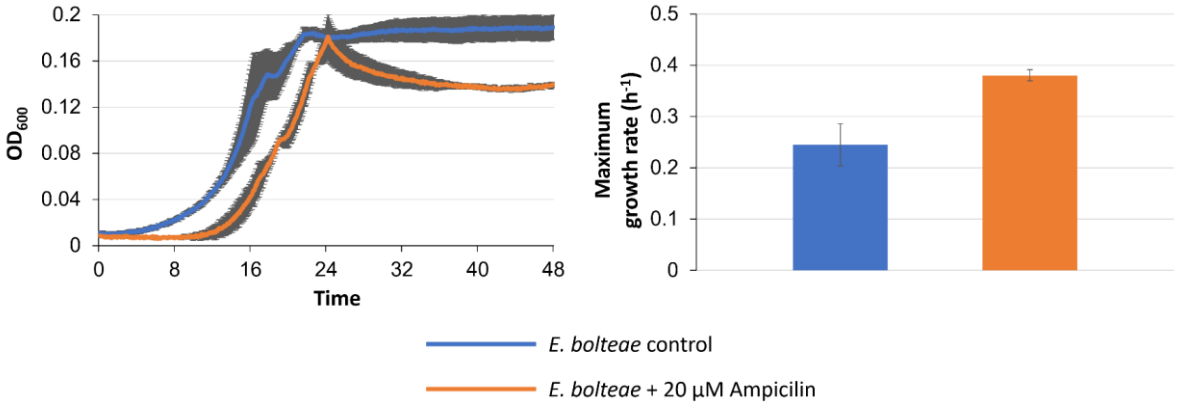


Figure 9: Comparison of growth curves and maximum growth rate of treated (20 µM ampicillin) and untreated *E. bolteae* DSM 15670. Significance: p = 0.0987268693890671

Another antimicrobial compound tested against selected bacterial strains was gentamicin. Gentamicin was used with a concentration of 20 µM, which led to an almost full growth inhibition of *P. vulgatus* DSM 1447 (Figure 10A). The growth rate of *E. coli* (human fecal isolate) was not significantly decreased by gentamicin, however, a significantly lower OD_{600} was observed compared to the control group (Figure 10B). No effect on the growth of *A. putredinis* DSM 17216 was detected (Figure 10C).

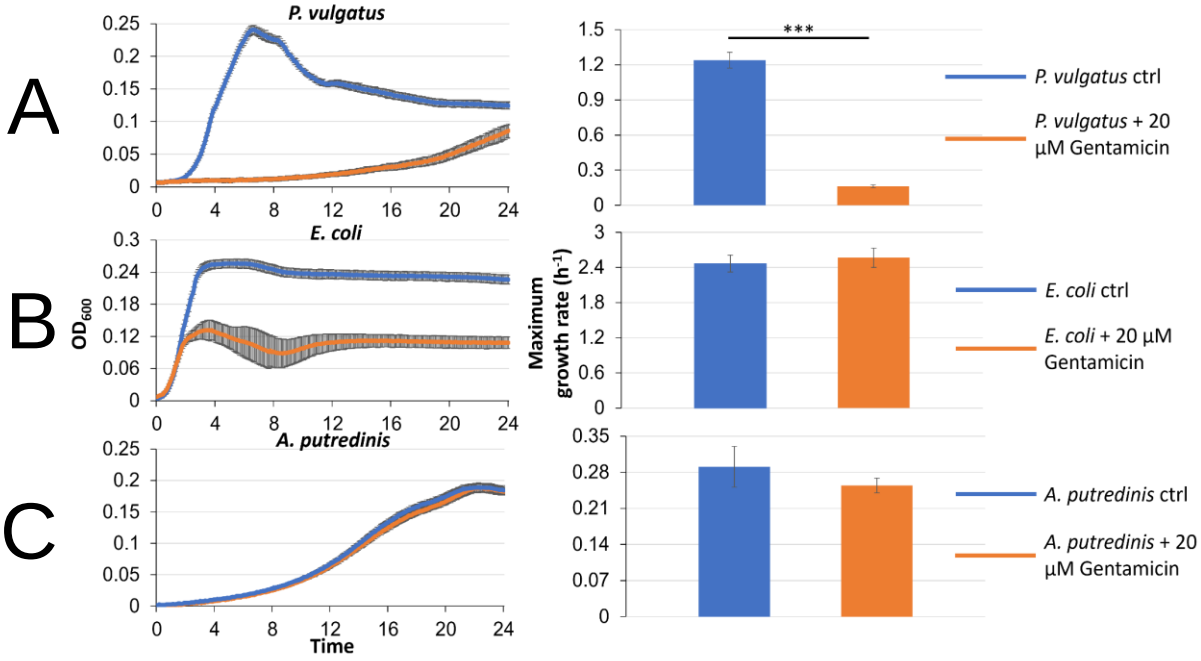


Figure 10: Growth curves and calculated maximum growth rate of three bacterial strains: A) *A. putredinis* DSM 17216. B) *E. coli* (human fecal isolate). C) *P. vulgatus* DSM 1447 with and without the addition of 20 µM gentamicin. Significance: ***= p < 0.005

3.6. The solvent methanol had no effect on the growth of the bacterial strains

As the bacterial extracts were dissolved in methanol, it was tested, if the inhibitory effect of the extract was in fact based on the antimicrobial compounds within the extracts or if the methanol also has an inhibitory effect on the growth of the bacterial strains. For all of the tested species, the addition of 20 µl methanol did not have any significant effect on the growth of the strains (Figure 11).

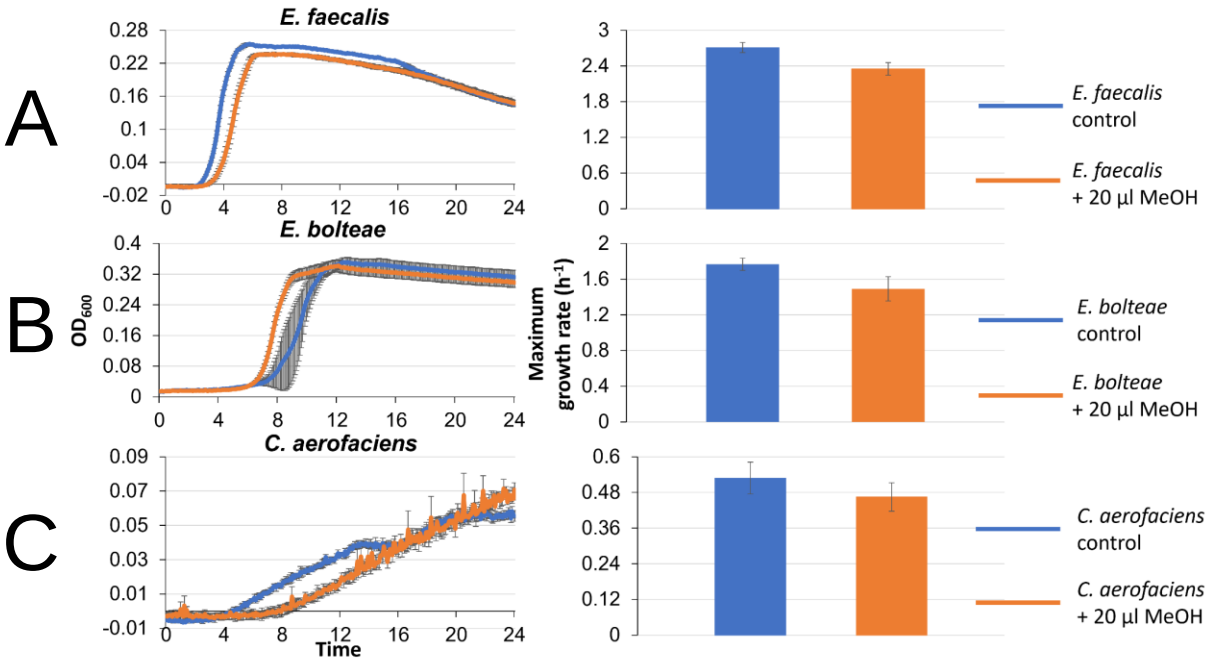


Figure 11: Comparison of growth curves and growth rates of A) *E. faecalis*, B) *E. bolteae* and C) *C. aerofaciens* with and without the addition of 20 µl MeOH. No significant differences between the treatment group and the controls could be detected.

3.7. *Streptomyces sp.* UV5 extract showed significant growth inhibition of selected gut bacterial strains

To screen for any effect of the bacterial extract derived from *Streptomyces* sp. nov. UV5 on the gut community, the extract was tested on a selection of gram-negative and gram-positive

strains. The addition of 20 µl UV5 extract showed a significant inhibition on the growth of *E. coli* (human fecal isolate). In comparison to the control (no addition of the extract), the growth was slower and a visible lag phase was observed. The maximum OD_{600} decreased significantly from 0.21 to 0.14 (Student's T-test: p = 0.0038). The strongest effect between the two treatments was observed for the calculated maximum growth rate, which led to a decrease in the growth rate from 1.3 $hours^{-1}$ to under 0.4 $hours^{-1}$ (Student's T-test: p = 0.0000039). The addition of 10 µl of the UV5 extract also showed a significant inhibition on the growth of *E. coli* (human fecal isolate), but no lag phase was observed. The maximum observed growth rate was 0.6 doublings per hour versus 1.3 doublings per hour (Student's T-test: p = 0.000015) in the control (Figure 12A). Testing the UV5 extract on *B. fragilis* DSM 2151 showed no significant effect on its growth (Figure 12B).

Testing the UV5 extract against gram-positive gut bacteria showed a strong inhibitory effect on their growth. The use of 10 µl of the extract was sufficient to completely inhibit the growth of *B. longum* subs. longum DSM 20219 and *E. bolteae* DSM 15670. Compared to the control group, doublings per hour decreased from 0.4 for *B. longum* subs. longum DSM 20219 (Student's T-test: p = 1.02E-07) and 0.3 for *E. bolteae* DSM 15670 (Student's T-test: p = 2.32E-07) to nearly no detectable growth rate (Figure 12C and 12D). For *E. faecalis* (human fecal isolate) no full inhibition of growth was observed, but the growth was significantly delayed, seen as lag phases for 4 and 10 hours when adding 10 or 20 µl of UV5 extract, respectively. The maximum growth rate was significantly reduced from 2 doublings per hour in the control to 1.4 (10 µl of extract; Student's T-test: p = 0.000083) or 0.6 (20 µl of extract; Student's T-test: p = 1.55E-11) doublings per hour (Figure 12E).

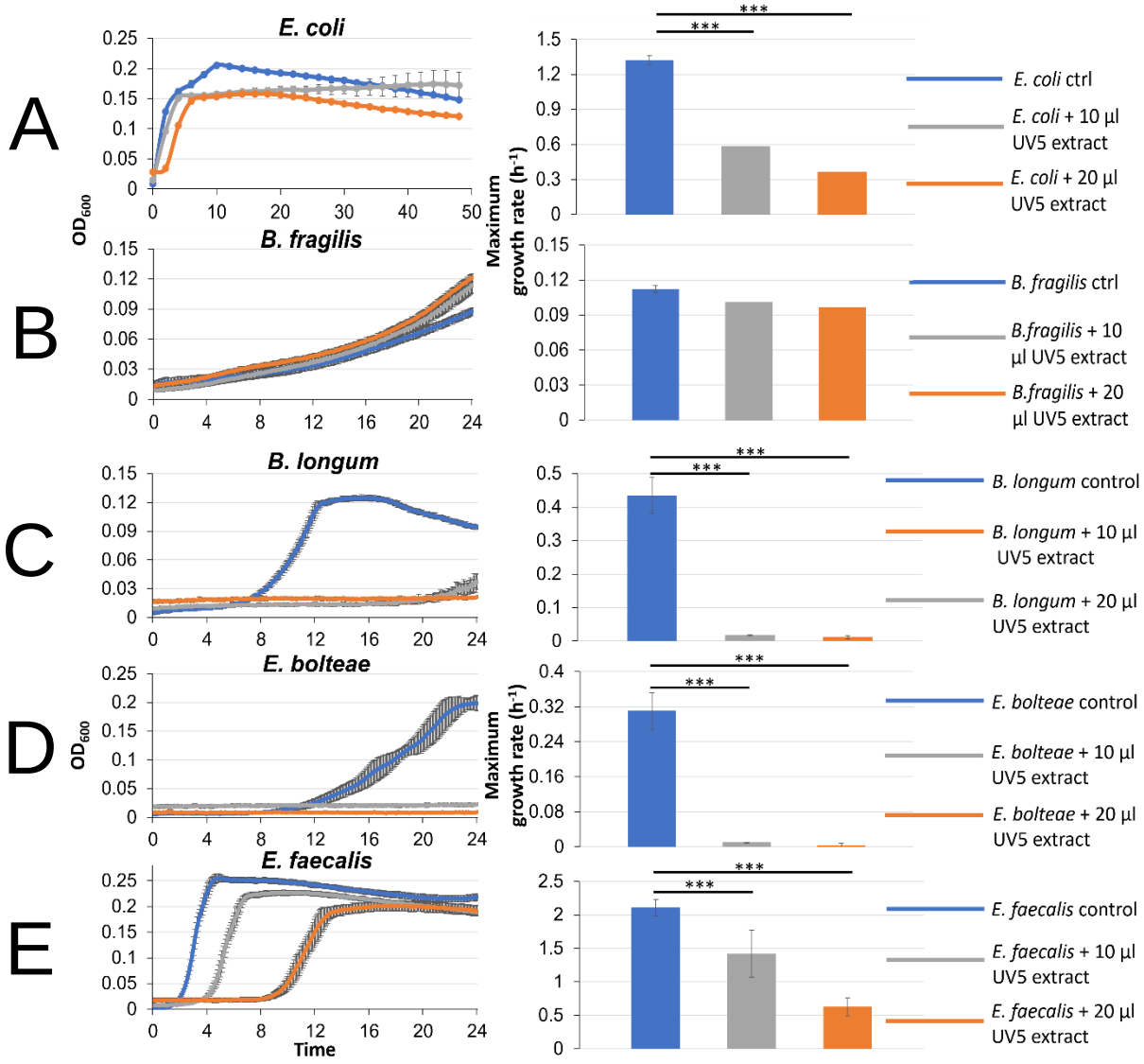


Figure 12: Bacterial extract from *Streptomyces* sp. nov. UV5 partly inhibited the growth of the tested gram-negative and gram-positive gut bacteria. A) Growth curves and maximum growth rate of *E. coli* (human fecal isolate). The maximum growth rate was significantly reduced when the extract was present. B) No significant effect of the extract on the growth curve as well as the maximum growth rate of *B. fragilis* DSM 2151 was observed. C) Full growth inhibition of *B. longum* subs. longum DSM 20219 by the extract. D) Full growth inhibition of *E. bolteae* DSM 15670 was observed when the extract was present. E) The extract led to a longer lag phase and reduced maximum growth rate in *E. faecalis* (human fecal isolate). Significance: ***= $p < 0.005$

Additionally, an upregulated culture-derived extract from *Streptomyces sp.* UV5 was tested against the same gut bacterial strains. It was shown in disc-diffusion assays that the extract has an higher antimicrobial activity against aerobically grown *E. coli* and *M. luteus* than the original extract (Kralova, unpublished).

The updated UV5 extract, where the production of the antimicrobial compounds was upregulated, showed a stronger inhibition against *E. coli* (human fecal isolate) and *B. fragilis* DSM 2151 compared to the original UV5 extract. For *B. fragilis* DSM 2151 the maximum growth rate was reduced from 0.76 doublings per hour in the control to 0.5 (Student's T-test: p = 5.70E-12) or 0.47 doublings per hour (Student's T-test: p = 9.51E-11) when 10 or 20 µl of the extract was added, respectively (Figure 13A). Furthermore, the growth inhibition of the upregulated UV5 extract on the growth of *E. coli* (human fecal isolate) was stronger compared to the original UV5 extract. The upregulated UV5 extract reduced the maximum growth rate to 0.16 doublings per hour (Student's T-test: p = 5.56E-14; Figure 13B). However, there was no increased inhibitory effect against *E. bolteae* DSM 15670 and *E. faecalis* (human fecal isolate) in comparison to the original UV5 extract (Figure 13C and D). Against *C. aerofaciens* (human fecal isolate), complete growth inhibition was observed by the addition of 10 and 20 µl of the modified UV5 extract. The maximum growth rate was decreased from 0.53 to nearly 0 (Student's T-test: p = 2.36E-08; Figure 13E).

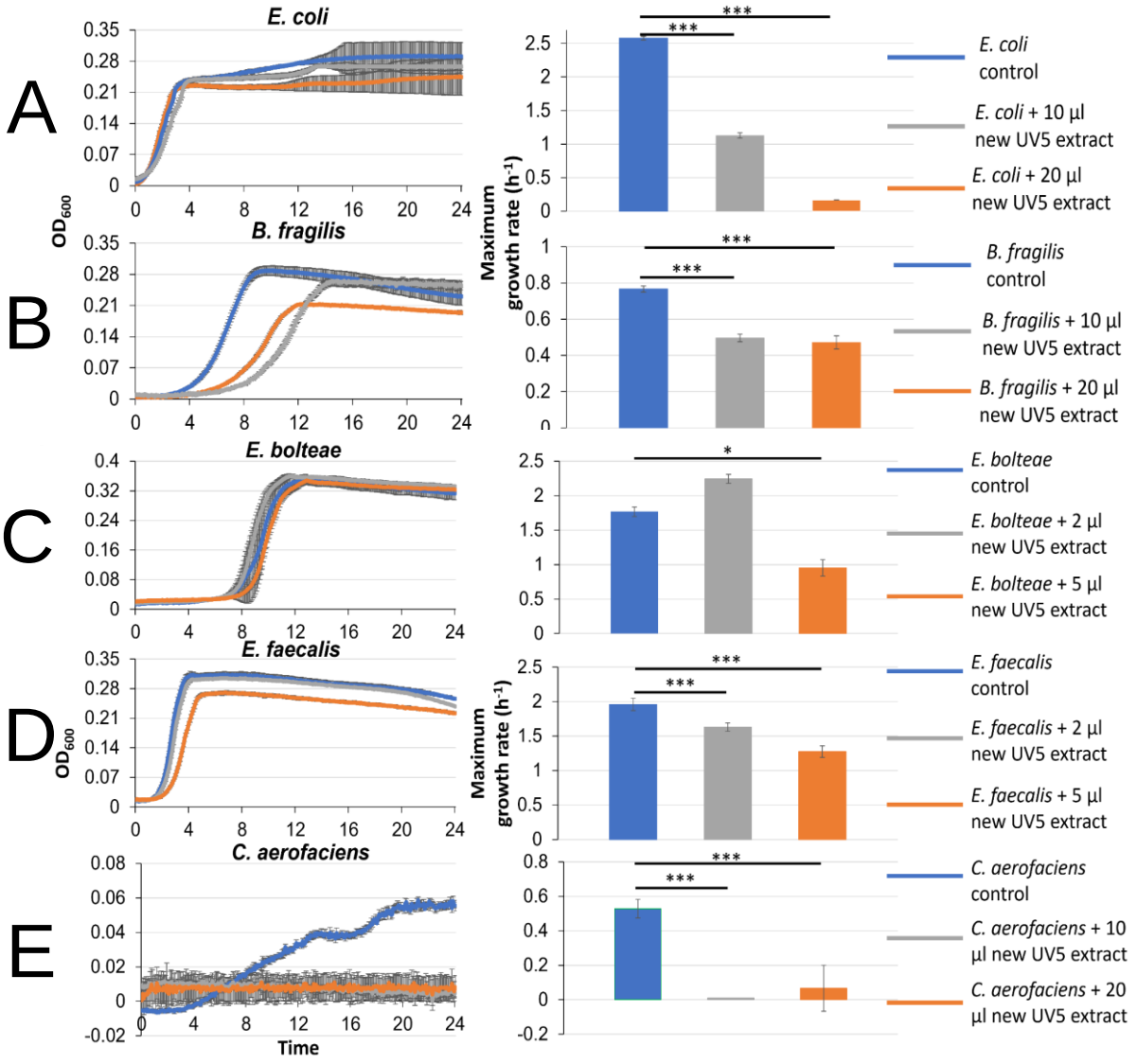
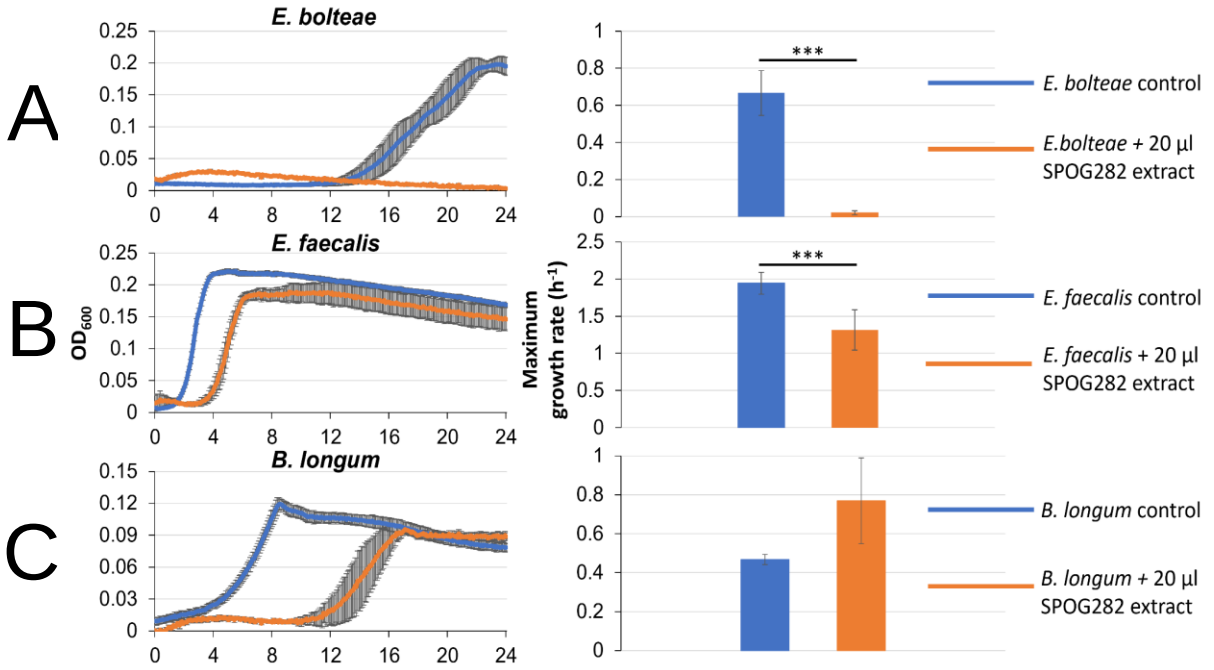


Figure 13: Upregulated culture-derived extract of *Streptomyces* sp. UV5 showed in parts an increased growth inhibition against the tested gut bacterial species compared to the original extract. A) The inhibitory effect on the growth of *E. coli* (human fecal isolate) was increased when compared to the original UV5 extract B). Growth curves and maximum growth rate of *B. fragilis* DSM 2151 showed a significant growth reduction compared to the original extract. C) No enhanced growth inhibition against *E. bolteae* DSM 15670 was observed. D) For *E. faecalis* (human fecal isolate) no difference was observed between the original and upregulated extract. E) Complete inhibition of growth of *C. aerofaciens* (human fecal isolate). Significance: *= $p < 0.05$ ***= $p < 0.005$.

3.8. The *Pseudomonas sp.* SPOG282 extract showed a significant inhibitory effect on the growth of different gut bacterial strains

Another bacterial extract, derived from *Pseudomonas* sp. SPOG282, was tested against different strains from the gut bacterial collection. Against *E. bolteae* DSM 15670 the extract from SPOG282 led to a full inhibition of growth of the bacterial strain (Student's T-test: p = 7.97-06; Figure 14A). For *E. faecalis*, a significant decrease in the growth was observed by the extract, the maximum growth rate was reduced from 2 to 1.3 doublings per hour (Student's T-test: p = 0.000059; Figure 14B). In contrast, no significant effect of the extract on the growth rate of *B. longum* subs. longum DSM 20219 was observed. Nevertheless, a strong lag phase was visible in the growth curves of *B. longum* subs. longum DSM 20219, where the exponential growth started after 12 hours in the treatment group versus 4 hours in the control group (Figure 14C). Furthermore, the SPOG282 extract was tested against *C. aerofaciens* (human fecal isolate) as well as *A. putredinis* DSM 17216. For both strains, a complete inhibition of growth was observed by the addition of 20 µl of extract (Figure 14D and E).



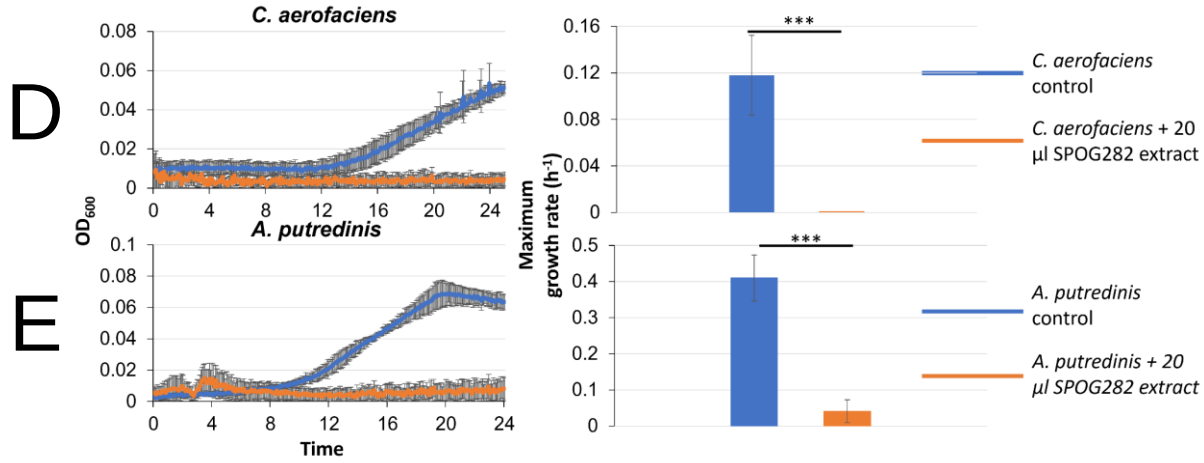


Figure 14: Effect of the bacterial extract from *Pseudomonas* sp. SPOG282 on the tested gram-negative and gram-positive gut bacteria. A) Full growth inhibition of *E. bolteae* DSM 15670. B) The growth of *E. faecalis* (human fecal isolate) was significantly reduced by the SPOG282 extract. C) No significant growth inhibition was observed for *B. longum* subs. longum DSM 20219 D) Full growth inhibition of *C. aerofaciens* (human fecal isolate) and E) *A. putredinis* DSM 17216. Significance: ***= $p < 0.005$.

IV. Discussion

Antibiotics are important contributors in the treatment of bacterial infections., but the overuse and misuse of antibiotics led to significant challenges, especially the rise of antibiotic-resistant pathogens. This highlights the need for the discovery of new antimicrobial compounds (Spellberg *et al*. 2013; Tsamo *et al*. 2021). However, antibiotics and other antimicrobial compounds not only kill pathogens but often target commensals of our human gut microbiome. This can lead to dysbiosis and disease (Weersma *et al*. 2020; Blaser 2016). In this thesis, crude metabolite extracts of *Streptomyces* sp. nov. UV5 culture and *Pseudomonas* sp. nov. SPOG282 culture were tested against different anaerobically grown gut species. This was done to extend the knowledge of the antimicrobial activity of the compounds in the extract from previously tested aerobic bacteria and pathogens to anaerobic gut commensals.

The two selected media (BHI and mGAM), which are typically used for the cultivation of anaerobic gut microorganisms, supported the growth of all the selected strains in this study. The media were selected based on previous studies, where BHI was shown to support stable growth of gut microbiome strains that were also used in this study (Aranda-Díaz *et al.* 2022; Celis *et al.* 2022). The medium mGAM has been additionally described as an appropriate medium for the cultivation of gut bacteria (Tramontano *et al.* 2018; Javdan *et al.* 2020). The second reason for using these two media was the time-effective preparation as they are both commercially available.

The protocol used in this study was developed for bacterial species that can grow in either BHI or mGAM growth media and in multi-well plates. Furthermore, the growth of the bacterial strains was monitored by OD measurements. Therefore, bacterial strains that form aggregates or precipitates cannot be used as the formation of any aggregates would interfere with absorbance measurements. After the first growth experiments, it was observed that the wells in the first and last column of the plates showed a significantly different growth pattern compared to the other wells. Bacterial growth in these two columns was slower and a steady decrease in the absorbance was recorded. The most likely reason for that was a higher evaporation of the cultures in these wells by uneven temperature distribution in the plate reader. Consequently, the first and last column of the plates were excluded from analysis as the results were not reliable. For all other wells, the reliability was confirmed by testing the growth of one selected bacterial strain of the community, which showed no significant differences for the growth parameters.

For testing crude bacterial extracts with potential antimicrobial activity or pure antimicrobial compounds against the tested strains, the extracts/antimicrobials must have certain properties.

They must be soluble and chemically stable in the growth media and incubation temperature. In addition, the color of the tested crude extracts/antimicrobial solutions should not interfere with the absorbance measurements. In this study, two antibiotics and three antimicrobial extracts generated by a *Streptomyces* and a *Pseudomonas* strain were used. All five amendments fulfilled the requirements for the inhibition experiments in the plate reader after being dissolved in respective solvents. The bacterial extracts used in the experiment were shown to have antimicrobial activity against gram-negative as well as gram-positive bacteria by classical disc diffusion assays on agar plates in previous experiments (Kralova, unpublished; Flieder, unpublished). This was only demonstrated under aerobic growth conditions, whereas the protocol for this study was developed for anaerobic growth in liquid media. However, the established approach is also applicable to aerobic growth conditions.

The analysis of the absorbance data from the plate reader was done using the R package 'growthcurver' as well as the Python package 'Anaconda'. Both were equally suited for the analysis, producing reliable and comparable values for the growth parameters calculation. Nevertheless, 'growthcurver' calculated more growth parameters and the handling of this tool was more user-friendly. Absorbance measurements were first done in two hour intervals. However, here the software had problems in fitting a logistic function to the OD measurements. As a consequence, the time intervals between the measurements were reduced to five minutes, which resulted in an improvement in calculating the maximum growth rate. Furthermore, the number of technical replicates was also increased from 4 to 10 for a higher statistical power, which on the downside slightly reduced the throughput. The generation of the growth curves as well as calculations of the growth parameters such as growth rate and generation time were done successfully for all strains. In summary, most of the strains could be grown in both, BHI and mGAM medium, however, a much better growth was observed in BHI medium for the majority of strains. For *P. copri* DSM 18205 and *R. intestinalis* DSM 14610, growth was only possible in the mGAM medium. It is likely that for some of the gut bacterial strains from the community BHI or mGAM were not the optimal medium for growth and faster growth with a higher OD_{600} might be reached in different growth media. To mention a few examples, for *B. fragilis* DSM 2151 Chopped Meat Medium or Columbia Blood Medium is suggested by DSMZ, for the *E. faecalis* human fecal isolate the recommended growth medium is Trypticase Soy Yeast Extract Medium or Columbia Blood Medium and for *L. hominis* DSM 23910 MRS medium is recommended by DSMZ. However, it would be too time-consuming to prepare for all species their recommended growth medium and therefore the use of BHI and mGAM were selected as convenient, pre-mixed media options, balancing ease of preparation with experimental reproducibility.

To test the overall protocol, two antibiotics were selected, ampicillin and gentamicin. Ampicillin is an extended-spectrum penicillin that is active against gram-positive as well as gram-negative bacteria including pathogenic ones. It is usually used for the treatment of respiratory and urinary tract infections as well as enteric fever and skin infections (Finch *et al.* 2003). It acts by the binding of the penicillin-binding proteins (PBPs), inhibiting the transpeptidase and thereby the synthesis of peptidoglycan is no longer possible, which eventually kills the cell (Tipper 1985). However, different bacteria have evolved numerous resistance mechanisms against this antibiotic, for example its degradation by expression of a β-lactamase or by modifications of the PBPs and thereby a decreased affinity to these proteins (Trevor & Katzung 2013; Klare *et al.* 2003). It was expected that ampicillin-sensitive and resistant strains would be observed in this study. The addition of 20 µM ampicillin had no growth-inhibiting effects on *E. bolteae* DSM 15670, which is in line with previous research (Maier *et al.* 2021). On the other hand, the same concentration of ampicillin fully inhibited the growth of *B. longum* subs. longum DSM 20219, the *E. faecalis* human fecal isolate and *L. hominis* DSM 23910. Bifidobacteria including *B. longum* have been shown to be sensitive to ampicillin (Kheadr *et al.* 2007), also enterococci like *E. faecalis* can be typically treated with ampicillin (Abele-Horn 2020) and lactobacilli such as *L. hominis* have also been shown to be sensitive to ampicillin (Ammor *et al.* 2007).

Gentamicin belongs to the group of aminoglycosides antibiotics and is especially active against gram-negative bacteria. It is often used to treat different kinds of infections including meningitis, peritonitis and ventriculitis (Tumialán *et al.* 2006; Wang *et al.* 2014; Barnes *et al.* 2003). Gentamicin binds to the 16S rRNA of the 30S ribosomal subunit leading to the disturbance of mRNA translation and thereby to truncated and non-functional proteins (Chaves & Tadi 2024). Nonetheless, among different bacteria resistances arose through numerous mechanisms including degradation or transport of the compound as well as the mutation or modification of the ribosomal target (Garneau-Tsodikova & Labby 2016). The addition of 20 µM gentamicin showed no growth inhibition for *A. putredinis* DSM 17216, which is in line with what is reported in the literature (Hugon *et al.* 2013). The *E. coli* human fecal isolate was significantly growth inhibited by gentamicin as a much lower OD_{600} in comparison to the control group was observed. In other studies, most of the *E. coli* isolates showed susceptibility to gentamicin (Abera & Kibet 2011; Daoud *et al*. 2020). Against *P. vulgatus* DSM 1447 a nearly full growth inhibition by 20 µM of gentamicin was observed. Interestingly, literature reports on susceptibility of *P. vulgatus* only against amoxicillin, imipenem and metronidazole, but not against gentamicin (Kierzkowska *et al.* 2020).

After the protocol and workflow were successfully established with the known and pure antibiotic compounds ampicillin and gentamicin, bacterial extracts from cultures of *Streptomyces sp*. UV5 and *Pseudomonas sp.* SPOG282 were tested. Notably, the

concentration of the antibiotic compounds in the bacterial extracts was not known at the time of this study. Therefore, different volumes of the extracts were used to reveal potentially dose-dependent inhibitory effects. Previous disc diffusion assays showed that the extract of *Streptomyces* sp. UV5 contained inhibitors against the growth of gram-negative as well as gram-positive bacteria (Kralova, unpublished). Here, it was shown that the UV5 extract had antimicrobial activity against the anaerobically grown gram-negative bacterium *E. coli* (human fecal isolate), as well as the gram-positive bacteria *B. longum* subs. longum DSM 20219, *E. bolteae* DSM 15670, and *E. faecalis* (human fecal isolate). However, only the growth of *B. longum* subs. longum DSM 20219 and *E. bolteae* DSM 15670 were fully inhibited. *E. coli* and *E. faecalis* (fecal isolates) showed reduced growth but not full growth inhibition. A three-hour and nine-hour lag phase was observed for the human fecal isolates *E. coli* and *E. faecalis*, respectively. Additionally, the growth was significantly slower compared to the control. A possible explanation could be a stress response of the bacteria, where they try to adapt to the antibiotic by activating defense mechanisms. There are several examples for that in the literature where the antibiotic triggers a change in gene expression, cellular metabolism and protein activity. This can lead to a decreased membrane permeability, an increased activity of efflux pumps or a change in the metabolic bypass which all reduce the possible action of the antimicrobial compound (Dawan&Ahn 2022; Viveiros *et al.* 2007). As the concentration of the antimicrobial compound within the UV5 extract was unknown, it is likely that the concentration was sub-lethal and therefore higher concentrations are needed for a full inhibition of the growth of *E. coli* and *E. faecalis* (human fecal isolates). Additionally, an upregulated extract of the *Streptomyces* sp. UV5 strain was tested. It was shown in disc diffusion assays that this extract had a higher antimicrobial activity compared to the standard UV5 extract (Kralova, unpublished). Therefore, it was expected that also the effect against the anaerobically grown gut bacterial strains would be enhanced. For *E. bolteae* DSM 15670 and *E. faecalis* (human fecal isolate), no increased inhibition by the upregulated extract was observed. An explanation could be that for these species the maximum inhibition was reached. In this case, the antimicrobial compound most likely acts as a bacteriostatic and the species are not killed compared to bactericidal antibiotics. The inhibitory effect of the upregulated extract on *E. coli* (human fecal isolate) was stronger compared to the original extract. This is likely attributed to the enhanced production of the antimicrobial compound. It is therefore plausible that the extract contained a higher amount of the bioactive compound and thereby the defense mechanism of *E. coli* (human fecal isolate) could be overcome. The original UV5 extract did not show any inhibitory effect against *B. fragilis* DSM 2151. However, the upregulated extract significantly inhibited the growth of *B. fragilis* DSM2151. This suggests that this strain is in principle not resistant to the antimicrobial compounds in the extract as previously thought, but a higher

concentration of the compound in the extract was essential to inhibit the strain *B. fragilis* DSM 2151.

The inhibitory activity of the *Pseudomonas sp.* SPOG282 crude extract against gram-negative bacteria like *P. putida* and gram-positive bacteria like *M. luteus* was demonstrated in previous experiments by disc diffusion assays (Flieder, unpublished). By mass spectrometry four yet unknown compounds were identified in higher concentrations. Two of these compounds, a lasso-peptide and a siderophore, were hypothesized to be the most likely candidates responsible for the antimicrobial effect of the extract (Flieder, unpublished). Lasso-peptides are natural products from the group of ribosomally synthesized and post-translationally modified peptides (RiPPs) and have a broad range of actions including antimicrobial, antiviral and antitumor activities (Salomón& Farías 1992, De Clercq 2000, Um *et al.* 2013).

As the concentration of the antimicrobial compound in the SPOG282 extract was not known, 20 µl of the extract was tested. This amount was used as it has been shown in the previous experiments with the UV5-derived crude extract that this volume was sufficient for the inhibition of the growth of most of the tested gut bacterial strains. Again, as the extract did show antimicrobial activity against gram-negative and gram-positive bacteria in the previous aerobic experiments, both groups were tested. As the growth of anaerobically gram-negative (*A. putredinis* DSM 17216), as well as gram-positive (*E*. *bolteae* DSM 15670 and *C. aerofaciens* human fecal isolate), was fully inhibited, the extract was demonstrated as active against both bacterial groups, in accordance previous experiments under aerobic conditions (Flieder, unpublished). A significant growth reduction, but no full inhibition was observed against the *E. faecalis* human fecal isolate. One reason is an inadequate concentration of the extract. Therefore a higher concentration of the antimicrobial compound may be needed to overcome the defense mechanisms of this strain against the compound. A second reason could involve only partial activity of the compound against *E. faecalis* (human fecal isolate) resulting in resistance of this strain against antimicrobial compounds in the extract. The growth rate of *B. longum* subs. longum DSM 20219 was not decreased by the antimicrobial compounds in the SPOG282 extract, nevertheless, the lag phase was longer. Most likely the strain had to adapt to the compound and thereby grew slower, however a normal growth of *B. longum* subs. longum DSM 20219 was observed later.

In this study a semi-high-throughput protocol for the anaerobic growth analysis of an established bacterial gut commensal collection was established. However, the protocol had some limitations that could be improved for further studies. To increase the throughput of the protocol, fewer replicates of the control and the treatment group could be implemented, thereby more strains can be analyzed in one 96-well plate reader-run. Furthermore, also bacteria from other human body habitats like the skin could be tested to explore a wider effect of the

antimicrobial compounds. Additionally, different antimicrobial compounds and other chemicals could be analyzed in one run. For example, in two studies by Lisa Maier, they monitored the effect of 144 antibiotics and in addition over 800 human-targeted drugs on the growth of 38 different gut commensals (Maier *et al.*2018; Maier *et al.* 2021). Also, it would be interesting to test potentially harmful pollutants that derive from human activities in the environment, and what their effects are on bacterial strains or communities (Singh *et al*. 2022).

The impact of the compounds on the growth of gut bacterial strains could also be tested in further studies beyond the analysis by optical density in the 96-well plate reader. There are various alternative new techniques like isothermal microcalorimetry, nanomotion-based measurements or the LifeScale instrument. In nanomotion-based techniques, they measure nanometric scale oscillations induced by atomic-force microscope cantilever. When the organisms are sensitive to a specific compound the amplitude of the cantilever oscillation is significantly reduced (Kasas *et al*. 2021). In isothermal microcalorimetry heat produced by metabolic processes of the organisms is measured and a growth inhibition by chemicals can be analyzed by a decreased heat production (Baldoni *et al.* 2009). They are characterized by a high rapidity as well as sensitivity and only a small number of cells are needed which makes it applicable also for very slow-growing organisms. However, these methods are limited in throughput and also are not well-tested under anaerobic conditions (Vasala *et al.* 2020; Kasas *et al.* 2021). The LifeScale instrument's microfluidic sensor measures the masses of thousands of individual bacteria and thereby rapidly detects antimicrobial susceptibility (Snyder *et al.* 2024). Additionally, promising would be the use of techniques like droplet-based microfluidics, which reduce the test volume to an absolute minimum. The measurement then can also occur via absorbance measurements. This method would vastly increase the throughput, scalability, and its ability to run parallel experiments, on the other hand this method could be difficult to implement and specific microfluidic chips are needed (Yu *et al.* 2022; Zhang *et al.* 2020). Furthermore, not only the effect of the compound on a single strain could be tested, but also the effect of chemicals on whole microbial communities because thereby whole community effects of the compounds like cross-resistance and cross-sensitivity can be studied.

In summary, a protocol for the semi-high throughput anaerobic screening of antimicrobial compounds on the growth of single bacterial strains of a gut commensals collection was established during this thesis. For the first time, the antimicrobial activity of extracts of the *Streptomyces* sp. nov. UV5 culture and the *Pseudomonas* sp. nov. SPOG282 culture against different anaerobically grown gut species was demonstrated. This includes the gut commensals *A. putredinis* DSM 17216, *B. fragilis* DSM 2151, *B. longum* subs. longum DSM 20219, *C. aerofaciens* (human fecal isolate), *E. bolteae* DSM 15670, *E. coli* and *E. faecalis* (human fecal isolates), which were all significantly inhibited in their growth by one or both

extracts. The compounds responsible for observed antimicrobial activities are currently being analyzed in collaboration with the Mass Spectrometry centre of the University of Vienna.

Zusammenfassung

Das Darmmikrobiom ist ein wichtiger Faktor für die menschliche Gesundheit. Veränderungen im Mikrobiom stehen im Zusammenhang mit Darmerkrankungen wie entzündlichen Darmerkrankungen, aber auch mit extraintestinalen Krankheiten, wie kardiovaskulären Störungen. Ein Faktor, der die Zusammensetzung des Darmmikrobioms beeinflusst, sind Medikamente, die das Wachstum von Darmkommensalen beeinträchtigen. Das Screening auf potenzielle Nebenwirkungen dieser Substanzen auf das Darmmikrobiom ist ein wichtiger Aspekt der Präzisionsmedizin. In dieser Arbeit habe ich zunächst eine mikroplattenbasierte Kultivierungsmethode angepasst, um den Einfluss von Metaboliten und chemischen Verbindungen auf das anaerobe Wachstum repräsentativer menschlicher Darmbakterien zu untersuchen. Die Stammsammlung bestand aus 18 Bakterienstämmen, die die phylogenetische und metabolische Vielfalt des menschlichen Darmmikrobioms widerspiegeln. Nach erfolgreicher Kultivierung in ausgewählten Medien wurden Wachstumsparameter wie Wachstumskurven und maximale Wachstumsraten ermittelt. Im nächsten Schritt wurden die Effekte bekannter Antibiotika auf das Wachstum ausgewählter Darmbakterien analysiert. Anschließend wurden bakterielle Rohmethanolextrakte, die aus Kulturen von *Streptomyces* sp. UV5 und *Pseudomonas* sp. SPOG282 gewonnen wurden, an ausgewählten Stämmen der Gemeinschaft getestet. Die Stämme UV5 und SPOG282 sind vielversprechende Kandidaten für die Produktion neuartiger Antibiotika und wurden zuvor als antimikrobiell wirksam gegen aerob wachsende gramnegative und grampositive Bakterien nachgewiesen. In dieser Studie zeige ich, dass die hemmende Wirkung der Rohmetabolit-Extrakte dieser Stämme sich auch auf ausgewählte Darmkommensalen erstreckt, darunter *Alistipes putredinis*, *Bifidobacterium longum*, *Collinsella aerofaciens*, *Enterocloster bolteae*, *Escherichia coli* und *Enterococcus faecalis*. Dabei wurde nachgewiesen, dass die Extrakte antimikrobielle Verbindungen mit einem breiteren Wirkspektrum enthalten können.

Glossary

Abbreviation	Meaning
antiSMASH	Antibiotics and Secondary Metabolite Analysis Shell
AUC	Area under the curve
BGCs	Biosynthetic gene clusters
BHI	Brain-Heart Infusion
BSA	Bovine serum albumin
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphate
DOME	Division of Microbial Ecology
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
eSNaPD	Environmental Surveyor of Natural Product Diversity
ExoSAP	Exonuclease I Shrimp Alkaline Phosphatase
F-primer	Forward primer
LE	low electroendosmosis
mGAM	Modified Gifu Anaerobic media
MIBiG	Minimum Information about a Biosynthetic Gene cluster
MQ	Milli-Q water
mRNA	Messenger ribonucleic acid
NRPs	Non-ribosomal peptides
OD	Optical density
PBPs	Penicillin-binding proteins
PCR	Polymerase chain reaction
PY	Peptone Yeast
RiPPs	Ribosomally synthesized and post-translationally modified peptides
R-primer	Reverse Primer
rRNA	Ribosomal ribonucleic acid
SMURF	Secondary Metabolites Unique Regions Finder
TBE	Tris-Borat-Ethylenediaminetetraacetic acid
YCFA	Yeast Casitone Fatty acids

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ChatGPT was used for editing of the English language.

Supplementary figures and tables

Supplementary Table S1: Incubation times for the different bacterial strains for the growth measurement in the 96-well plate reader (^{T}=type strain).

Bacterial strain	Incubation time in the plate reader
A. muciniphila DSM 22959^T	24 hours
A. putredinis DSM 17216^T	24 hours
A. rectalis DSM 17629	24 hours
B. caccae DSM 19024^T	24 hours
B. fragilis DSM 2151^T	24 hours
B. longum subs. longum DSM 20219^T	24 hours
B. ovatus DSM 1896^T	24 hours
B. thetaiotaomicron DSM 2079^T	24 hours
B. wadsworthia LT0012/SQ01	48 hours
B. xylanisolvens DSM 18836^T	24 hours
C. aerofaciens human fecal isolate	24 hours
E. bolteae DSM 15670^T	24 hours
E. coli isolate from human feces	24 hours
E. faecalis isolate from the hospital	24 hours
L. hominis DSM 23910^T	24 hours
P. copri DSM 18205^T	48 hours
P. vulgatus DSM 1447^T	24 hours
R. intestinalis DSM 22959^T	48 hours

	Time	Blank	A_putredinis_1	A_putredinis_2	A_putredinis_3	A_putredinis_4	A_putredinis_5
1	175	0.1023684	0.1108	0.1145	0.1097	0.1121	0.1162
2	180	0.1038526	0.1139	0.1165	0.1135	0.1156	0.1163
3	185	0.1033421	0.1147	0.1182	0.1129	0.1152	0.1187
4	190	0.1039737	0.1142	0.1197	0.1159	0.1188	0.1206
5	195	0.1048105	0.1186	0.1209	0.1172	0.1196	0.1242
6	200	0.1046263	0.1183	0.1257	0.1176	0.1215	0.1229
7	205	0.1040053	0.1190	0.1240	0.1177	0.1234	0.1250
8	210	0.1036842	0.1204	0.1266	0.1184	0.1236	0.1260
9	215	0.1037474	0.1229	0.1257	0.1227	0.1261	0.1274
10	220	0.1024316	0.1229	0.1270	0.1238	0.1266	0.1287
11	225	0.1037053	0.1259	0.1302	0.1276	0.1310	0.1325
12	230	0.1036895	0.1325	0.1334	0.1273	0.1313	0.1346
13	235	0.1042211	0.1317	0.1359	0.1321	0.1338	0.1383

Supplementary Figure S2: Example (*A. putredinis*) for the input data for growth parameter calculation using the R package 'Growthcurver'. The first column contains the incubation time, the second column the absorbance of the blank measurement (media alone), followed by the absorbance measurements of all the replicates of the bacterial strain.