The microbiome of the burying beetle *Nicrophorus vespilloides* as an untapped source for the screening of bioactive small molecules

INAUGURAL-DISSERTATION

zur Erlangung des akademischen Grades

Dr. rer. nat.

der Naturwissenschaftlichen Fachbereiche

der Justus-Liebig-Universität Gießen

Vorgelegt von

Philipp Heise, Master of Science

Gießen, im Januar 2019

1. Gutachter **Professor Dr. Andreas Vilcinskas**

Institut für Insektenbiotechnologie

Fachbereich Agrarwissenschaften, Ökotrophologie und Umweltmanagement

Justus-Liebig-Universität Gießen

2. Gutachter **Professor Dr. Thomas Wilke**

Institut für Tierökologie und Spezielle Zoologie

Fachbereich Biologie und Chemie

Justus-Liebig-Universität Gießen

„It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body. The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant.“

Alexander Fleming (1881-1955)

Table of contents

Summary

Nicrophorus vespilloides is a native species of burying beetles, the reproduction of which depends on small vertebrate carcasses e.g. those of small rodents. Carrion is buried is buried in the soil in order to reduce competitition with other scavenger insects. However, this behaviour unevitably increases the exposition of the carrion to a broad range of soil-borne microorganisms. Therefore, beetles of the genus *Nicrophorus* have evolved an efficient strategy for carcass preservation, thus to preserving the carrion as food source for its larvae.

It appears, however, that the phenomenon of carcass preservation by burying beetles is one of nature's best kept secrets. A number of pioneering studies have shown that oral and anal secretions of *N. vespilloides* contain antimicrobial and preserving and repellent molecules. Notably, beetles of the genus *Nicrophorus* harbour a unique gut flora, which is applied onto the surface of the carcass in order to contribute to its preservation.

This study focusses on the cultivation of bacterial isolates originating from the gut flora and the antimicrobial compounds produced by those microbes. Therefore, *N. vespilloides* caught in the wild as well as laboratory-hatched animals were investigated. Beetles were dissected, their gut was removed, and gut-associated microorganisms were cultivated. Pure cultures obtained by this procedure were identified by 16*S* rRNA gene analysis.

To identify producers of antimicrobial activities, a classical screening for natural products was performed. Approximately 800 extracts were subsequently tested against a broad spectrum of taxonamically defined bacteria and fungi. In the course of this screening, a multitude of antimicrobial activities could be observed.

Some isolates of the highly abundant genus *Serratia* displayed a broad spectrum of antimicrobial activity. A single isolate, which could be identified as *Serratia marcescens*, effectively inhibbite *Staphylococcus aureus*. This strain was selected for further detailed studies. The antibiotic activity was isolated and its structure elucidated by NMR spectroscopy. Finally, the antibiotic principle was identified as serrawettin W2. After purification, this cyclic pentadepsipeptide exhibited antimicrobial activity against further Gram-positive bacteria. Serrawettin W2, which has previously been reported as a nematode repellent, was shown to act as a nematostatic agent. The combination of these antimicribial and nematostatic bioactivities could be of importance for the ecology of burying beetles. After burying, the carcass is not only threatened by microorganisms but also by soil-borne nematodes. Consequently, a symbiosis of *Nicrophorus* sp. with a beneficial gut bacterium is hypothesised to positively contribute to to the preservation of the carcass, which is used for reproduction of the beetles.

Zusammenfassung

Der Totengräber *Nicrophorus vespilloides* ist ein heimischer Käfer, der zur Reproduktion das Aas kleiner Wirbeltiere, v. a. Nager, benötigt. Das Vergraben des Kadavers dient der Vermeidung von Konkurrenz mit anderen necrophilen Arthropoden. Dieses Verhalten führt jedoch zwangsläufig zur Exposition des Kadavers gegenüber einer mannigfaltigen, bodenbürtigen Mikrobenflora. Daher sind Käfer der Gattung *Nicrophorus* auf eine hoch effiziente Konservierungsstrategie angewiesen, um den Kadaver vor unkontrollierter Verwesung zu schützen und ihren Larven als Nahrung zugänglich zu machen.

Diese Konservierung ist ein bislang kaum verstandenes Phänomen in der Natur. Erste Untersuchungen zeigten jedoch, dass im Oral- und Analsekret von *N. vespilloides* antimikrobielle, konservierende und repellente Substanzen enthalten sind. Darüber hinaus verfügt er über eine ungewöhnliche Darmflora. Diese wird auf die Oberfläche des vorgefundenen Kadavers aufgebracht und trägt höchstwahrscheinlich zu dessen Konservierung bei.

Ziel dieser Studie war die Kultivierung von bakteriellen Isolaten aus der Darmflora des Totengräbers *N. vespilloides* und deren Untersuchung auf die Bildung antimikrobieller Naturstoffe. Dazu wurden sowohl Wildfänge, als auch im Labor gezüchtete Exemplare untersucht. Die Tiere wurden seziert, der Verdauungstrakt entnommen und die darmassoziierten Mikroorganismen mit verschiedenen mikrobiologischen Methoden kultiviert. Die erhaltenen Darmisolate wurden auf Basis des 16*S* rRNA Gens identifiziert.

Zur Auffindung von Antibiotikaproduzenten, erfolgte ein klassisches Naturstoff-Screening. Etwa 800 der gewonnenen Extrakte wurden anschließend in Bioaktivitätstests gegen ein umfangreiches, aus taxonomisch definierten Bakterien- und Pilzkulturen bestehendes Spektrum von Testkeimen geprüft. Hierbei wurde eine große Vielfalt an antimikrobiell aktiven Extrakten vorgefunden.

Einige Isolate der häufig isolierten Gattung *Serratia* zeigten breite antimikrobielle Aktivität gegen die Testorganismen. Ein Isolat, welches als *Serratia marcescens* identifiziert werden konnte, hemmte *Staphylococcus aureus* und wurde für eine vertiefende Bearbeitung, d.h. Isolierung und anschließende Strukturaufklärung des antibiotischen Prinzips mittels Kernresonanzspektroskopie (NMR), ausgewählt. Dieses konnte schließlich als Serrawettin W2 identifiziert werden. Das aufgereinigte zyklische Pentadepsipeptid zeigte antimikrobielle Wirkung gegen weitere gram-positive Testkeime. Für das in der Literatur als Repellent gegenüber Nematoden beschriebene Serrawettin W2 wurden außerdem nematostatische Eigenschaften nachgewiesen. Die Kombination dieser antimikrobiellen und nematostatischen Bioaktivitäten könnte von Bedeutung für die Ökologie des Totengräbers sein. Da der Kadaver durch das Vergraben nicht nur Mikroorganismen ausgesetzt ist, sondern auch Nematoden, könnte die Symbiose mit einem solchen Darmbakterium maßgeblich für den Erfolg der Konservierung des als Brutstätte genutzten Kadavers sein.

Introduction

2.1 Insects - a treasure chest of nature

Insects are the most diverse group of organisms in the animal kingdom. More than 70% of all validly described, recent animal species known so far are members of the class of insects (**Figure 1**) (Chapman, 2009). However, recent estimates account for up to 5-6 million insect species, approximately 20% of which have scientifically been described and classified to date (Groombridge, 2002). In the course of evolution, insects have colonised almost every possible habitat, including even those that appear as hostile to life at first sight such as arctic and desert environments. Their ability to adapt, even under harsh environmental conditions, made them the most successful group of animals on earth. Consequently, an impressive number of insect species have evolved highly sophisticated physiological, biochemical, and behavioural adaptations and strategies in order to utilise otherwise inaccessible ressources.

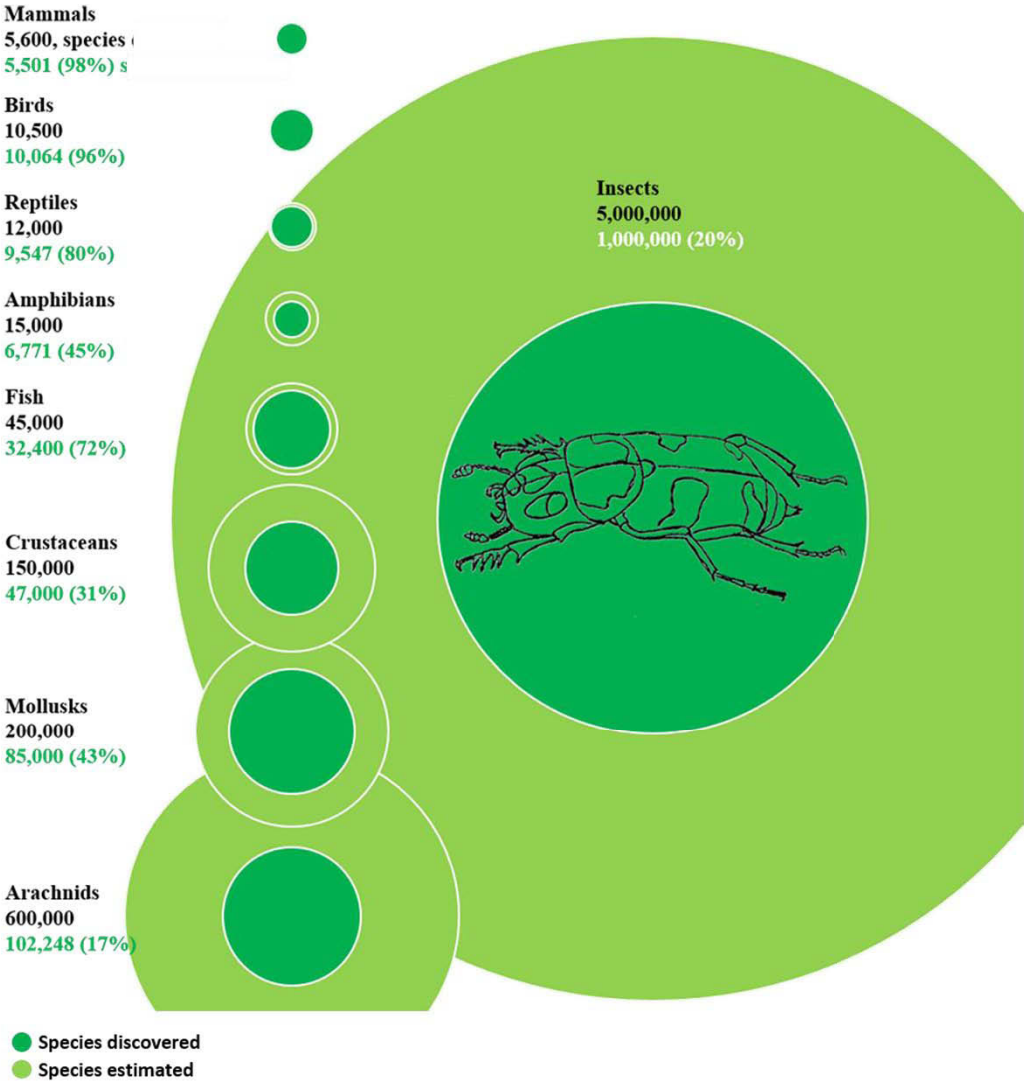


Figure 1: Animal diversity on earth according to Chapman (2009).
The figure shows the biodiversity on earth, indicating the abundance of insects, the most successful but yet a relatively less explored group of the animal kingdom.

The Antarctic midge *Beldica antactica* and the Sahara Desert ant *Cataglyphis bicolor* are often referred to as paradigms of insects from extreme habitats. *B. antactica* is the largest land-living animal permanently residing in Antarctica, and is able to withstand the extreme cold (Lee et al., 2006). In contrast, *C. bicolor* is well-adapted to the extreme heat of the Sahara and survives temperatures constantly ranging above 50 °C in the daytime, which are lethal to most of the other animal species (Sherwood, 1996).

Beside extreme habitats, unusual food sources are also utilised by insects. Keratin, the main protein of wool and feathers, for example, is an extremely stable material, which can only be digested by a rather limited number of insects and bacteria. The clothes moth *Tineola bisselliella* is able to feed on clothes or feathers, thus using keratin as a nutrient source (Day, 1951). Other insects, *e*. *g*. the larvae of the black soldier fly *Hermetia illucens*, even utilises oily food, manure and other organic matters that are hardly biodegradable. Thus, this insect may be developed into a perfect solution for the rising global waste problem. Notably, several applications in bioconversion have already been reported (Sheppard et al., 2002). Another example for an outstanding insect life style is the burying beetle *Nicrophorus vespilloides*, which depends on vertebrate carcasses for reproduction (Pukowski, 1933).

2.2 The burying beetle *Nicrophorus vespilloides*

N. vespilloides is a widespread species of burying beetles across Northern America and Europe. It can regularly be found all over Germany. This beetle is 12-18 mm in size and belongs to the family of Silphidae. At present, the genus *Nicrophorus* includes 68 species with unique strategies for reproduction and parental care (Sikes et al., 2008).

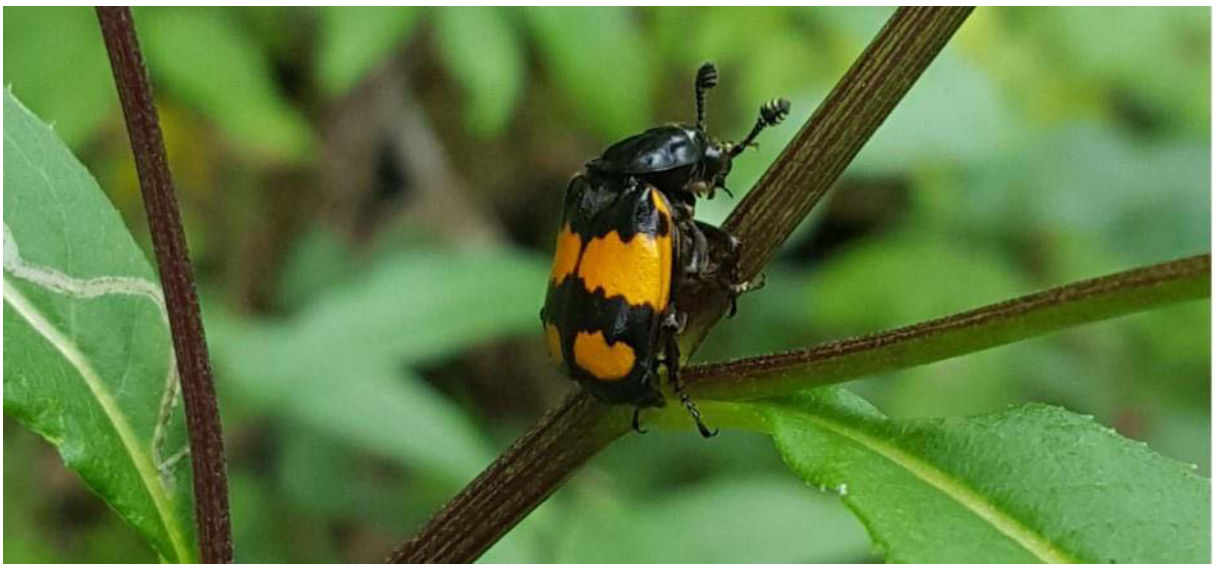


Figure 2: *N. vespilloides* with its black and orange elytra.
The picture was taken at the Hoherodskopf (Vogelsberg)/Germany.

Adult *N. vespilloides* hunt for dipteran larvae but cadavers of small vertebrates, preferably rodents, are necessarily required for reproduction. The beetle perceives the odour of carrion over long distances and locates the carcass where mating takes place after a couple of burying beetles has successfully conquered and defended the carrion against competing arthropod scavengers (Pukowski, 1933). To effectively protect the carrion against competitors and scavengers, *N. vespilloides* is burying the carcass. This, on the other hand, leads to different problems. Burying a rich nutrient source such as a dead animal in the ground exposes it to a very high microbial load of soil- borne bacteria, fungi and nematodes. This scenario normally accelerates the decay of the carcass (Vogel et al., 2017). In order to preserve the carrion, the beetle uses its own oral and anal secretions that are applied to the surface after the hairs or feathers of the animal have been removed (Hall et al., 2011). This process is thought to promote the spread of secretions on the shaved carcass surface.

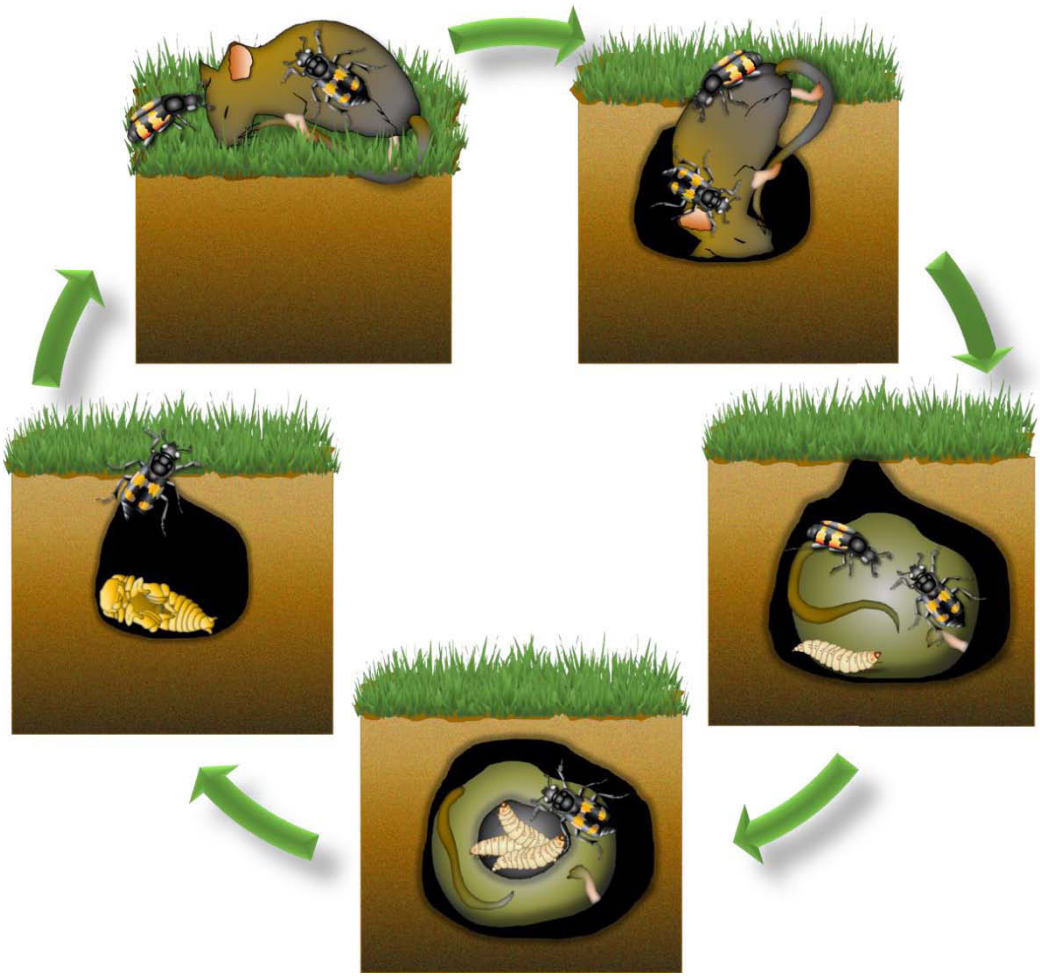


Figure 3: Simplified life cycle of *Nicrophorus vespilloides*.
The burying beetle's life cycle is initiated by the detection of a carcass, which triggers the mating of the couple. Mating takes place on the carcass, which then will be shaved. After applying oral and anal secretion on the surface, the carrion it is buried in the ground. Eggs are laid in the close proximity of the brood ball, and the freshly hatched larvae are guided to the processed brood ball. Once inside, the larvae are initially fed by the adults – a phenomenon that is referred to biparental care. In addition, both parents contribute to the continuous preservation of crypt and brood ball. Finally, 3rd instar larvae laeve the brood ball, and after a migratory phase they return to the soil in order to pupate. Drawing kindly provided by Dr. Henrike Schmidtberg.

After preservation, the carcass is buried in the ground. This highly sophisticated process turns the carrion into a round brood ball (Pukowski, 1933; Milne and Milne, 1976). The brood ball is then stored in the so-called crypt and eggs are laid in the close proximity (Pukowski, 1933). After hatching, the larvae are guided into the brood ball and feed from the pre-digested food inside whilst the parents take care of the larvae as well as carcass preservation and defence. Up to now, little is known about the preservation process on a molecular level. Studies unveiled the increase of lytic activity in the secretions during the presence of a carcass (Cotter and Kilner, 2010) and the upregulated expression of antimicrobial peptides (AMPs) (Jacobs et al., 2016).

2.2.1 Anatomy of the burying beetle

Most of the body of the burying beetle *N. vespilloides* is coloured in black but the elytra carry four squares that are lively coloured in orange. The small head is covered by an extremely hard cuticle and is equipped with strong mandibles and two highly evolved club-like antennae. The sensilia of these antennae are specialised to detect sulphuric volatiles in low quantity. This helps the beetle to locate carrion by its odour (Kalinova et al., 2009). Behind the head, the pronotum represents a solid chitin shield, which forms a strong functional unit with the head.

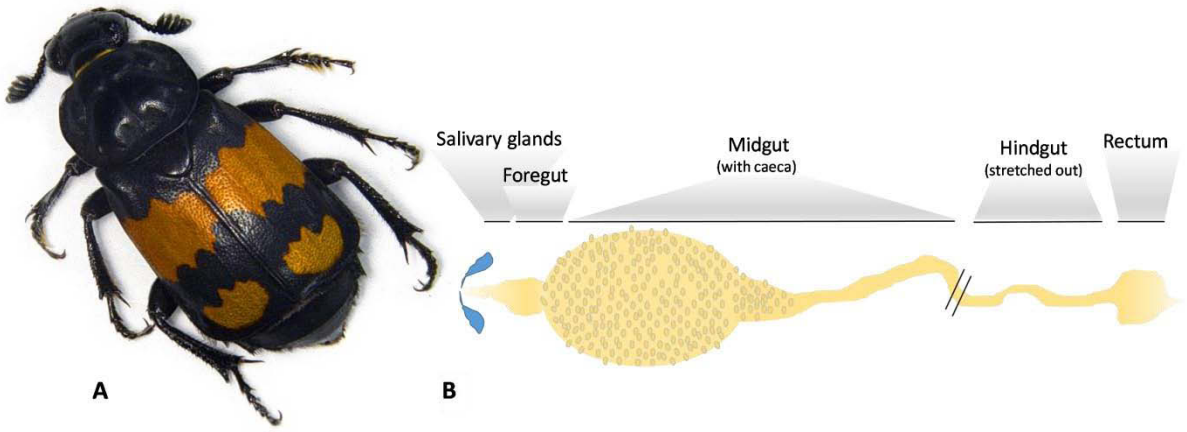


Figure 4: Anatomy of the burying beetle and its gut.
A: *N. vespilloides* and its characteristic appearance
B: Scheme of the gut compartments and the salivary glands. For graphical reasons, the hindgut has been shortened but it is the longest part of all gut segments.

The most remarkable internal organ of the beetle is its gut, which can be subdivided into four compartments. The oral opening represents the first part of the foregut. The latter opens to form a voluminous part, the midgut, which is of mesodermal origin. It ends up in a bottleneck-shaped narrower part. The thick part of the midgut is covered by caeca (Vogel et al., 2017) and joins into the hindgut, which is formed by ectodermal tissue. The coiled hindgut is a thin and flat compartment, being the longest of all gut segments. Anatomically, it is located on top of the midgut. The long hindgut

terminates in the rectum close to the anal abdominal part, often referred to as the anal bladder. Most of the microbial gut flora is harboured in the area between the midgut and the rectum.

2.3 Microbiome studies

The question behind the mechanisms of carcass preservation by burying beetles has been addressed by different authors. Recently, the upregulation and secretion of immune-related AMPs by the beetles was taken into account (Jacobs et al., 2016). However, the hitherto neglected contribution of the highly specialised microbial gut community of *N. vespilloides* to the biosynthesis of simple disinfecting, carcass-preserving metabolites was also suggested (Degenkolb et al., 2011; Shukla et al., 2018).

The first cultivation-independent studies of male and female *N. vespilloides* beetles using pyrosequencing uncovered the microbial community (Kaltenpoth and Steiger, 2014). The microbiomes of both male and female hindgut did not differ much and were dominated by the phyla of *Gammaproteobacteria*, and *Firmicutes*. *Alpha-* and, *Betaproteobacteria* as well as *Bacteriodetes* and *Actinobacteria* were less dominant (Vogel et al., 2017). *Enterobacteriaceae*, *Xanthomonadaceae* as well as *Enterococcaceae* and the phylum *Firmicutes* were identified as the most abundant bacterial taxa. A remarkable feature of *N. vespilloides* is the presence of the yeast genus *Yarrowia* in the rectum. The *Yarrowia* species found seem to be unique for this species of burying beetle; however, their role still remains hypothetical. Recent, ongoing investigations have indicated a possible contribution of *Yarrowia* sp. to the metabolic decomposition of the two malodorous, putrefaction-associated diamines cadavarine (1,5-pentanediamine) and putrescine (1,4-diaminobutane) (Brinkrolf et al., in preparation).

Insects often host a unique community of microbes, which play a key role for digestion and metabolism of the harbouring organism (Rajagopal, 2009). Hence, this facilitates insects to utilise diverse nutrient sources. This connection has also been appreciated by the applied biotechnology, which therefore focuses on mining of insect gut microbiomes as sources for new species of microorganisms, enzymes, proteins, and secondary metabolites (Krishnan et al., 2014; Piel et al., 2004). The primary focus is laied on cellulose and xylan hydrolysis for biofuels (Warnecke et al., 2007) or vitamin production (Akman et al., 2002). Furthermore, the challenge of antibiotic resistance has been addressed and novel antibiotic compounds have been identified, for example, a β-lactam from the midgut microbes of the gypsy moth *Lymantria dispar* (Allen et al., 2009).

2.4 Natural product research

The application of medical plants is very common among all cultures and indigenous tribes. The knowledge about such "natural pharmacies" is often age-old (Wohlleben et al., 2016); nevertheless is still used to formulate remedies that have not only been praised in folk medicine, but also serve as modern pharmaceuticals. Nowadays the most notable difficulty is, amongst others, the discovery of bioactive substances with novel modes of action to meet the demands of modern medical applications. For more than 200 years, plants and microorganisms have been used as a prolific source of purified, pharmaceutically active secondary metabolites, which were then used as drugs (Li and Vederas, 2009). These secondary metabolites are still of high value for modern drug discovery and the pharmaceutical industry.

Secondary metabolites are organic molecules synthesised by most, if not all living organisms such as microbes, algae, corals, sponges, plants and lower animals (Maplestone et al., 1992). They are not involved in the primary development of the organism itself. In contrast to primary metabolites, which are crucial for the development of the species, secondary metabolites support, for example, the survival of their producers by acting as an immune defensive (Agostini-Costa et al., 2012). Therefore, secondary metabolites are of interest to pharmaceutical research and can still be considered as a prolific source of new drugs.

2.4.1 Antibiotic drug discovery - the past

About 200 years ago, Friedrich Sertürner marked the beginning of modern drug discovery by isolating morphine as a pain killer from the seed pods of immature poppy (*Papaver somniferum*). Morphine is the main alkaloid of crude opium, which has been known and applied as a pain killer for millennia (Hamilton and Baskett, 2000).

Until 1928, drugs were mainly derived from higher plants. In autumn of 1928, Alexander Fleming accidently discovered the antibiotic effects of a substance, later called penicillin, which has produced by the mould *Penicillium rubens* (Houbraken et al., 2011) growing on a blood-agar plate with staphylococci (Fleming, 1929). By the time of World War II, penicillin became the first industrially produced antimicrobial drug on the Allied side, which satisfied the urgent need for a potent anti-infective.

After World War II, the pharmaceutical research initiated the massive screening of soil microorganisms due to Fleming´s pioneering discovery. Those screening campaigns required the acquisition of bacteria, which were mostly screened for the inhibition of human pathogens (bioactivity-guided drug discovery). Companies like Eli Lilly, Novartis or Höchst asked their employees to collect soil samples on their trips around the world to find new antibiotic-producing bacteria, actinomycetes, or fungi. This global sampling led to the discovery of numerous valuable antibiotics from soil-borne bacteria, mainly

Streptomyces sp. (Katz and Baltz, 2016). These days, such sampling approaches are prevented by the Nagoya protocol for the protection of genetic resources of a country (Soares, 2011).

In the early decades of drug discovery, bioactivity-guided-screenings and flask fermentations were the limiting factors resulting in a screening capacity of 35.000 strains per year at Eli Lilly's reported by Richard H. Baltz. Due to high success rates in *Streptomyces*, screening was mainly focused was on filamentous *Actinobacteria*.

Progress in screening techniques and physico-chemical structure elucidation led to a higher throughput. Moreover, the exploitation of novel sources for natural products, for example marine bacteria and algae, promoted the discovery of new drugs. During the past two decades, the upcoming availability of DNA cloning and sequencing, has remarkably accelerated modern approaches in natural products discovery of the 21st century.

By the end of the so-called 'Golden Age' of antibiotic drug discovery in the 1970s, "low hanging fruits were harvested" and it became more and more difficult to make new discoveries (Baltz, 2007). This is also represented in the timeline of antibiotic discoveries (see **Fig. 5**), which clearly indicates a gap from 1987 until present (Lewis, 2012).

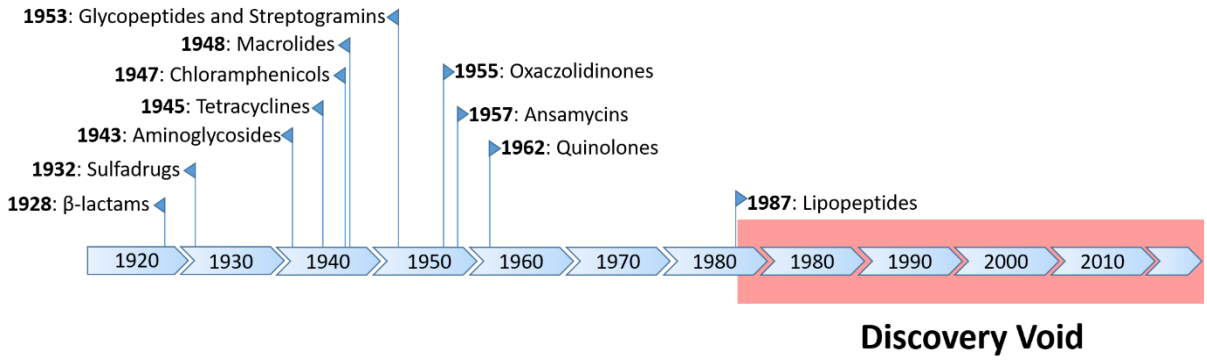


Figure 5: Discovery timeline of antibiotic classes.
This timeline illustrates the discovery of the most important classes of antibiotics during the last 90 years. The time from 1928 until the end of the 1970s is commonly referred to as the "Golden age of antibiotic drug discovery" because of the abundant findings made in these decades. With the last new class, the lipopeptides, being discovered in 1987, we are now facing a three decades void of newly approved antibiotic classes.

To overcome this lack, combinatorial chemistry was propagaed in the 1990s, which aimed at screening of large chemical libraries containing synthetic substances by high-throughput approaches. The principle of combinatorial chemistry is the generation of chemically synthesised molecules in large varieties and the screening of those molecule libraries for antimicrobial compounds (Navre, 1998). Despite the fact that the concept was extensively promoted, the scientific outcome and the value for the pharmaceutical industry were close to nothing. This was mainly because these artificially generated compounds were devoid of the chemical diversity that is known from natural products (Feher and Schmidt, 2003). This failure led to the conclusion that mimicking the characteristic features

of naturally occurring antibiotic compounds is a decisive step to introduce target-oriented chemical modifications (Newman and Cragg, 2007).

As soon as genetic tools became more and more inexpensive in the late 1990s and early 2000s, techniques such as whole genome sequencing led to the rapid discovery of a lot more of secondary metabolite gene clusters in *Streptomyces* genomes (Bentley et al., 2002; Baltz, 2014) than previously expected. Notably, only a limited number of all biosynthetic gene clusters (BGC) are expressed under standard laboratory conditions. This insight broadened the options for triggering and manipulating of silent BGCs (Bode et al., 2002) and led to the modern approaches of antibiotic drug discovery.

2.4.2 Antibiotic drug discovery - the present

Approximately only 1% of all bacteria on earth can be cultivated and stored under laboratory conditions. Consequently, the discovery of new bacterial taxa is a challenging and demanding, but necessary, inevitable task (Pham and Kim, 2012). Nevertheless, the availability of genomes marked the decisive change in drug discovery techniques (Katz and Baltz, 2016).

Due to the common availability and the decreasing expense of genetic techniques, the focus in drug discovery shifted towards genomics-based approaches. *Streptomyces* spp., for example, have been screened for antibiotic secondary metabolites from the beginning of natural products research. Nevertheless, a large set of BGCs were additionally discovered by genome analyses. Therefore, genome mining is currently regarded as the key technology of the modern antibiotic drug discovery (Zerikly and Challis, 2009). More and more BGCs, such as the well-known polyketide synthases (PKS) and non-ribosomal peptide synthetase (NRPS) clusters, are unraveled, thus opening up the field for genetic work such as cluster cloning and overexpression of these constructs (Cimermancic et al., 2014). Different attempts of direct cluster activation are currently considered techniques. Usually, chemicals or stress during fermentation, are applied but also genetic strategies such as overexpression of regulators, gene cluster duplication or synthetic biology (Baltz, 2016; Piel, 2011). Still, these techniques do not seem very potent for industrial large-scale drug discovery approaches.

Until the year 2002, more than 22,000 bioactive secondary metabolites have been published with 90% of all compounds originating from microorganisms. Roughly 55% of these are derived from fungi, ~ 20% from the bacterial genus *Streptomyces*, ~ 10% from rare *Actinobacteria*, and ~ 15% from other bacteria (Berdy, 2005).

Nevertheless, recent studies corroborated the decisive importance of classical microbiology besides all modern, genome-based techniques. Recently, R. Müller and colleagues published on the correlation of the taxonomic distance between members of the well-studied group of *Myxobacteria* and the chemical diversity. It was concluded that a larger genetic distance between secondary metabolite-producing strains increases the probability of finding novel chemical structures (Hoffmann et al., 2018).

By leaving the classically explored taxa aside, the focus is to be redirected to the cultivation of hitherto neglected bacteria (Lewis et al., 2010). Consequently, symbiotic microorganisms appear as a yet underexplored but highly promising, source of new secondary metabolites (Piel, 2004).

2.4.3 Antibiotic resistance

Antibiotic resistances are on the rise (Bush et al., 2011; Schäberle and Hack, 2014). According to recent reports of the World Health Organisation (WHO), antibiotic resistance is one of the biggest threats to global health. Therefore, people become increasingly aware of the problems approaching due to the misuse of therapeutic antibiotics in human medicine, animal farming, and agriculture (Witte, 1998; McManus et al., 2002).

However, it should be kept in mind that antibiotic resistance is a naturally occurring phenomenon in bacterial communities (American-Academy-of-Microbiology, 2009; Cavanagh et al., 2016). Some bacteria are using secondary metabolites such as antibiotics to outcompete others and resistances may help to win this arms race (Waksman and Woodruff, 1940). Antibiotics used in medication make their way into the environment, which significantly contributes to the uncontrolled spread of resistances all over the world (Allen et al., 2010). When human pathogens become resistant not only to standard antibiotics but also to antibiotics of last resort, we are running out of options. According to the WHO, this alarming process is already progressing. Multiply resistant bacteria are on the rise, which results in increasing numbers of severe infections, especially in hospitalenvironments.

For example carbapenem, a β-lactam drug of last resort, which had been the weapon of choice against multidrug resistant (MDR) bacterial infections for a long time, is becoming increasingly ineffective due to spread of resistance (Potter et al., 2016).

All of these facts underline the importance of antibiotic drug discovery and the urgent need of a scientific renaissance of this research field. By the end of the 1990s, most of the large pharmaceutical companies suspended their research for new antibiotics, because of the immense costs and comparatively low revenue expected (Lewis, 2012). Therefore, it is high time to resolve this problem by private-public partnerships, as in the case of neglected tropical diseases. Alliances of academia, big pharmaceutical companies are necessarily required to promote the antibiotic drug discovery (Schäberle and Hack, 2014). The recently established Fraunhofer-Sanofi (Evotec) partnership in Gießen is a paradigm of a joint antibiotic drug discovery approach for the future.

The aim of the project

The burying beetle *Nicrophorus vespilloides* may serve as an intriguing, hitherto unexplored source to study food conservation and the production of antimicrobials associated with it. Furthermore, it is a potential model organism for knowledge-based research in the field of anti-invective natural products. The preservation of carrion requires a highly specialised chemical ecology. Such an extraordinary lifestyle can be of immense value for target-oriented screening of pharmaceutically active secondary metabolites. Therefore, it is of decisive importance to study the gut microbiota of the beetle and to investigate a possible role of the microbiome for carcass preservation.

Aim 1: isolation, cultivation and identification of the gut microbes

A fundamental part of the project is the cultivation of microbes from the beetles' gut. The dissection of the beetle leads to the accessibility of the gut. Microbiological techniques have to be applied to cultivate as many gut microbiota as possible. In order to find suitable conditions for microbial growth, different media and cultivation conditions have to be tested. The identification of the microbes will be achieved by 16*S* gene amplification and Sanger sequencing. The establishment of a strain collection of gut microorganisms is crucial for further work.

Aim 2: fermentation, chemical extraction and antimicrobial screening

The obtained microbial isolates have to be de-replicated bioinformatically by 16*S* gene sequences. Selected isolates will then be cultivated. Liquid cultures on rotary shakers will be performed to maximise the chance of BGC induction by nutrient limitation. Chemical extraction and bioactivity testing will be performed according to special operation procedures of the Fraunhofer-Sanofi cooperation to include the obtained material in the daily testing routine. The antimicrobial screening will be performed in the high throughput setup of the Sanofi facility. In parallel, the test procedures will also be established for in-house use.

Aim 3: Analytic and isolation of interesting candidates

The positive tested extracts from the primary antimicrobial screening have to be analysed by high-performance liquid chromatography (HPLC) and mass spectrometry. Therefore, crude extracts will be separated by fractionation, and bioactivity tests will be repeated to finally identify the active fractions. For identification and dereplication of the screened natural products, mass spectrometry will be used in combination with the natural products database AntiBase. If a hit remains unknown, the compound will be isolated and its structure will be analysed by nuclear magnetic resonance spectroscopy (NMR). This will also require a scale-up of the fermentation process and the chemical isolation.

Methods

4.1 Insects

Nicrophorus vespilloides beetles were either caught in the wild during the warm period in Giessen (Germany) or bred in captivity by H. Vogel (Max-Plank Society, Jena, Germany). The wild beetles can be found from May to the middle of September. The temperature is therefore the limiting factor, nights with temperatures below 10°C and days with hot weather (> 27°C) were considered as unsuitable for catching.

The wild beetles were caught by placing a dead mouse (*Mus musculus*) on the soil surface in a modified container with a lid featuring a hole of ~3 cm in diameter. The container was then buried with the lid at ground level and checked daily. The volatiles of the decaying carcass allured the beetles from distance, which then crawled into the container where they were trapped. Furthermore, *N. vespilloides* was tried to be hatched in captivity. For this, a so called *Faunarium* (Exo Terra/HAGEN Deutschland GmbH & Co, Holm, Germany) was filled with soil from the habitat and humidified by spraying. The beetles were provided with a dead mouse to induce the mating. Successful breeding was investigated but humidity issues prevented the transformation from pupae to adults. The rearing therefore was stopped after several trials.

4.2 Dissection and sample preparation

Before dissection, the beetles were cooled in the fridge to reduce movement and then washed sequentially in 1% bleach, water, 70% ethanol and again in water for 1 min each for surface sterilisation. Subsequently, the beetles then were dissected in phosphate buffered saline (PBS) and the gut was removed. An open reaction tube with PBS buffer was located next to the dissection and was used as a control for airborne contamination. Gut sections were crushed in 1x PBS buffer using a 1000 µL pipette tip and each preparation was serially diluted and plated on brain heart infusion (BHI), trypticase soy as well as trypticase soy yeast extract (TSB), each supplemented with 1.5% agar.

4.3 Cultivation

The isolated bacteria were cultivated on brain heart infusion broth (BHI) agar, potato glucose agar (PGS) or tryptic soy broth (TSB) agar for 1–4 days. Beside complex media, 1.5% water-agar was also used for cultivation for up to 3 weeks. Antibiotic-resistant cultures were selected on media supplemented with 50 µg/mL ampicillin and 25 µg/mL kanamycin. All cultures were primarily incubated at 28°C. In case of rapid growth, the temperature was reduced to 21°C (room temperature) and/or the nutrient yield was lowered by 50% for enhanced colony selection (e.g. for the genus *Proteus*). Colonies were visually

selected, picked and streaked out onto fresh agar until no contamination was detected by microscopy. Isolates were then kept in Roti®-Store cryo vials at –80°C for long-term storage.

For fungal cultivation, yeast extract glucose chloramphenicol agar (YGC) was used. This agar is selective for fungi and eliminates bacterial growth by the use of chloramphenicol. Cultivation was performed in the same manner as described before.

4.3.1 Media

brain heart infusion (BHI) broth (Agar)	pig brain infusion	7.5 g/L
	pig heart infusion	10 g/L
	peptone	10 g/L
	glucose	2 g/L
	sodium chloride (NaCl)	5 g/L
	disodium phosphate	2.5 g/L
	pH-value	7.4 ± 0.2
	(agar)	(15 g/L)
tryptic soy broth (TSB)	casein peptone	17 g/L
	soy peptone	3 g/L
	d(+)-glucose	2.5 g/L
	sodium chloride (NaCl)	5 g/L
	K_2HPO_4	2.5 g/L
	pH-value	7.3 ± 0.2
	(agar)	(15 g/L)
yeast glucose chloramphenicol (YGC) Agar	yeast extract	5 g/L
	glucose	20 g/L
	chloramphenicol	0.2 g/L
	pH-value	6.6 ± 0.2
	(agar)	15 g/L
potato glucose agar (PGS)	potato extract	4 g/L
	desxtrose	20 g/L
	pH-value	5.6 ± 0.2
	(agar)	15 g/L
Mannitol Salt Agar	lab-lemco' powder	1.0
	peptone	10.0
	mannitol	10.0
	sodium chloride	75.0
	phenol red	0.025
	ph-value	7.5 ±
	agar	0.2
		15.0

4.4 Identification

4.4.1 Bacterial colony PCR

The bacterial isolates from the *N. vespilloides* gut were identified by 16*S* rRNA gene amplification and Sanger sequencing (Eurofins, Germany). Therefore, the commonly used 16*S* primer set 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-ACC TTG TTA CGA CTT-3') were chosen (Lane, 1991; Masschelein et al., 2013). Each amplification was based either on a standard colony PCR or was achieved by previously lysing the cells in 0.2% SDS (Packeiser et al., 2013) followed by a 10-fold dilution in PCR-clean water. For the standard colony PCR, cell material was picked using a pipette tip and mixed with 30 µL of double-distilled PCR-clean water before heating the cell suspension to 95°C for 5 min in a thermal block. Afterwards, 1-3 µL of the lysed cells were then used as the template.

The PCR comprised 32 cycles of denaturation (95°C, 5 min), annealing (56°C, 30 s) and extension (72°C, 1 min) followed by a final elongation at 72°C, 1 min before storage at 4°C. The PCR products were separated by agarose gel electrophoresis using 1.5% agarose gel at 110 V for 40 min, and product bands were isolated using the Qiagen Gene Jet gel extraction kit.

4.4.2 Eukaryotic colony PCR

The ITS1 and NL4 primer set was used to amplify a 1200 basepairs (bp) fungal DNA fragment. This amplicon is covering a terminal part of the 18*S* small ribosomal subunit, the whole Internal Transcribed Spacer (ITS) 1 and 2 as well as the 5.8*S* region in between and the beginning of the 28*S* large ribosomal subunit (**Figure 6**).

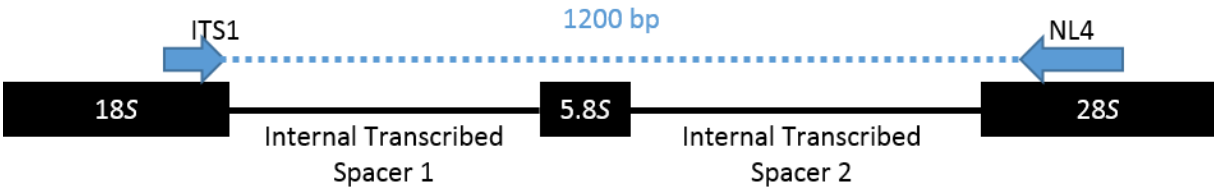


Figure 6: Binding site of the eukaryotic primer set.
The ITS1 primer binds to the 18*S* small ribosomal subunit region, the NL4 primer binds reverse to the antisense strain in the region of the 28*S* large ribosomal subunit. Both reads cover a distance of 1200 bp including the ITS1, the 5.8*S* region and the ITS2.

The colony PCR programme was initialised by denaturation for 10 min at 95°C, followed by annealing (55°C, 1 min) and elongation at 70°C for 2 min. It comprised 36 cycles end ended with a final elongation step of 5 min at a temperature of 70°C before storage at 4°C. Gel electrophoresis and gel extraction were performed as described above.

4.4.3 Genetic identification

Sanger sequencing was performed by Eurofins, Germany. Therefore, the Mix2Seq Kit of Eurofins was used. The obtained sequences were checked for high quality and then used for BLASTn search implemented into CLC Main Workbench 7. In parallel the forward and reverse reads were aligned and also BLASTn checked via the internal pipeline of the bioinformatics group (AG Goesmann/University of Giessen) by Sebastian Jaenicke.

For phylogenetic analysis, Mega 7 was used to build and display phylogenetic trees. After the alignment of 16*S* sequences from isolated bacteria with 16*S* type-strain references from the National Institute for Biotechnology Information (NCBI) with the CLC Main Workbench, a maximum-likelihood phylogenetic tree was generate with Mega 7.

4.4.4 Genomic DNA extraction

For whole genome PacBio sequencing of three selected bacterial isolates, genomic DNA (gDNA) isolation was performed using the Genomic tip 500/G and the Genomic DNA Buffer Kit from Qiagen. For the gDNA isolation, the strains were fermented in 100 mL media (media dependent on strain) within 300 mL Erlenmeyer flasks. The suspension was then centrifuged in two 50 mL reaction tubes at full speed for 3 min to sediment cells and the supernatant was discarded. Depending on the size of the pellet, either a single pellet or both pellets were used for gDNA isolation. The isolation was performed according to the Qiagen Genomic DNA Handbook.

4.5 Genome analysis

Whole genome sequencing was ordered at Macrogen Korea (Seoul, Korea). Genomic DNA was used to construct PacBio RS II SMRT libraries with an insert size of 20 kb. Whole genome sequencing was performed on a PacBio RS II instrument according to the supplier's standard practice. Genome assemblies were prepared using the Hierarchical Genome Assembly Process Pipeline (HGAP.3) including a read correction, a Celera-based assembly, and assembly polishing with Quiver (Chin et al., 2013). Circularization of bacterial genomes was achieved with Circlator (Hunt et al., 2015) using the PacBio sequencing reads. Prediction of plasmid sequences was achieved with PlasFlow (doi: 10.1093/nar/gkx1321). Phylogenetic classification of the genomes was calculated with REFERENCESEEKER (Oliver Schwengers, not yet published) in order to estimate the percentage of homologous sequence regions (conserved DNA) with the most closely related genome available from the public data bases and respective ANI (average nucleotide identity).

Gene finding and functional annotation was carried out in two steps using (i) Prokka (Seemann, 2014) and (ii) a reannotation involving a best BLAST hit method versus Swiss-Prot and Trembl (UniProt-Consortium, 2018) in order to improve the standard function annotation output from Prokka.

Subsequently, AntiSMASH was used for the prediction of secondary metabolite producing gene clusters (Blin et al., 2017).

4.6 Fermentation and chemical extraction

Preliminary to the fermentation process, pre-cultures of 30 mL within 100 mL Erlenmeyer flasks were incubated over night at 200 rpm and 28°C. One millilitre of the pre-culture was used for the inoculation of 100 mL media within 300 mL Erlenmeyer flasks with paper plugs. These flasks were then incubated for one, six and nine days and afterwards shell-frozen on liquid nitrogen in 1000 mL round bottom flasks. The shell-freezing provides a high surface ratio for increased freeze drying. After freeze drying, the resulted material was scraped of the flask wall and collected in 50 mL Greiner tubes. The lyophilised fermentation then was mixed with ~ 50 mL ethyl acetate (EtOAc) and ultrasonicated in an ultrasonic water bath for 15 min to open up the cells. Subsequently, the resulting EtOAc crude extract was filtered and collected in a fresh 50 mL tube. The folding filter with the remaining filtrate was then returned to the original Greiner tube and 50 mL of methanol (MeOH) was added. The ultrasonic treatment and filtering was repeated as described, resulting in crude MeOH extracts. These crude extracts were evaporated within a Speedvac. Here, the operation temperature was critical and should not exceed 35°C to prevent damage to the samples by heat. Afterwards, the dry samples were resolved in 5 mL MeOH and transferred to glass vials. Another evaporation and resolving step within one mL MeOH resulted in a 100 fold concentrated crude extract, which was stored at 4°C.

The described process was scaled up to obtain higher amounts of biological material for compound isolation. Therefore, culture volume was scaled up to 2000 mL each flask, incubated in 5000 mL Erlenmeyer flasks. Liquid-liquid extraction was performed with EtOAc for scaled up fermentation eperiments, but without a positive result.

4.7 Bioassay (growth inhibition assay)

4.7.1 Bioassay (Sanofi-FhG)

The activity tests were set up against clinicaly relevant strains using 10, 1, 0.5 and 0.25 µL of each crude extract tested in duplicates in 96-well plates to ensure high throughput. For *S. aureus*, *E. coli* or *C. albicans,* 100 µL cation-adjusted Müller-Hinton broth was used, inoculated with ~ $5x10^5$ cells/mL and incubated shaking (180 rpm) at 37°C for18 h. *M. smegmatis* was grown for 48 h at 37°C in BHI supplemented with 1% Tween 80. Afterwards, the cell suspension was diluted to $\sim 1\times10^5$ cells/mL in cation-adjusted Müller-Hinton broth. One hundred µL of the diluted suspension were dispensed on white 96-well flat bottom plates and incubated for 48 h at 37°C shaking at 180 rpm. Cell viability was determined by using the BacTiter-Glo assay (Promega, Germany) and a LUMIstar OPTIMA microplate luminometer (BMG Labtech, Germany) for the read-out (Dardic et al., 2017).

4.7.2 Bioassay (in-house)

For in-house fraction testing, it was mandatory to establish a liquid testing method for fast identification of active fractions after HPLC fraction collection as well as Flash purification.

Growth inhibition was measured against both positive and negative controls (gentamycin serial dilution/fluconazole) with *E. coli* D31 K12 and *Staphylococcus aureus* DSMZ 799 used as screening strains. Therefore, liquid cultures were grown to an optical density (OD_{600}) of 1 and subsequently diluted 1:500 with medium. A volume of 100 µL was used as inoculum per well. Cultivation was performed for 18 to 24 h within the separated and evaporated crude extract fractions. The read-out was performed by measuring the OD_{600} using a plate reader.

4.7.3 Nematode motility assay

Caenorhabditis elegans was grown on nematode growth medium (NGM) covered with a lawn of *E. coli* OP20 for 4 days at 20 °C. The worms were washed off the Petri dish into a 15-ml Falcon tube using a glass Pasteur pipette and M9 buffer. The nematode suspension was then centrifuged at 440 g for 2 min and the supernatant was removed. The pellet was washed again with M9 buffer and, after another round of centrifugation as above, the content of the tube was reduced to 3.5 ml. To eliminate the worms and synchronize the suspension, we added 1.5 ml of bleach mix (0.5 ml 5 M NaOH, 0.5 ml NaOCl and 0.5 ml water). The suspension was briefly mixed and shaken until the color of the solution changed from yellowish to clear (4–6 min). The tube was filled up to 15 ml with M9 buffer and centrifuged for another 4 min at 2760 g. The supernatant was quickly removed without touching the pellet until only 0.1 ml remained, and 15 ml of M9 buffer was added. In order to completely remove the bleach mix, the tube was carefully inverted and the pellet was rinsed three times with M9 buffer before centrifugation at 2760 g to remove all liquid. Finally, we added 10 ml of M9 buffer and 10 µl of cholesterol (5 mg/ml in 99% ethanol). Nematodes hatched overnight while the culture was shaking at room temperature. The nematode-containing medium was centrifuged for 4 min at 1200 g to remove all liquid. After a final washing step with 15 ml M9 buffer, the freshly hatched nematodes were centrifuged and the concentration was adjusted to 10 L1 nematodes per 100 µl by diluting with NGM seeding medium. The latter was prepared by supplementing 10 ml NGM with 10 µl 5 mg/ml cholesterol, 10 µl 25 mg/ml carbenicillin and 50 µl of an E. coli OP50 overnight culture.

The tests were carried out in triplicate in a 96-well plate with ivermectin (10 µg/ml in DMSO) as the positive control. Purified serrawettin W2 in DMSO was serially diluted from 256 to 2 µg/ml. DMSO was used as the negative control. Non-motile nematodes were counted under the microscope after 24 h. To test for nematicidal effects, the incubated suspension was diluted 1:10 in M9 buffer and seeded on NGM agar plates containing *E. coli* OP50. The Petri dishes were checked for vital nematodes after 4 days of incubation at room temperature.

4.8 Chemical Analytics

4.8.1 High-performance liquid chromatography (crude extract)

The crude extracts were fractionated by high-performance liquid chromatography (HPLC) using a Dionex ICS 3000 instrument fitted with a Dionex Acclaim 120 C8 column. The separation was carried out using solvent A (water plus 1% formic acid) and B (acetonitrile plus 1% formic acid) at a flow rate of 250 µL/min. The initial solvent ratio was 80%/20% A/B held for 5 min, increasing to 100% B over 55 min followed by a 15-min hold, finally the ratio was set to the initial condition for the duration of 1 min. The column was re-equilibrated for 15 min. Fractions were collected during the first 75 min. The injection volume was 10 µL of filtered crude extract. Active fractions were analysed by mass spectroscopy and the putative antimicrobial agents were screened against AntiBase (Wiley) for identification.

4.8.2 Large-scale purification by FLASH chromatography

For compound isolation, large-scale fermentation with up to 10 litre fermentation volume was extracted. The methanol crude extract was then treated multiple times with acetonitrile to precipitate proteins and to reduce the obtained crude extract. The precipitated proteins were filtrated and resolved in water. Afterwards the aquatious protein extract was freeze dried and then resolved in methanol with a concentration of 50 mg/mL. Both obtained fractions (MeOH and aquatious with a concentration of 50 mg/mL) were again tested for activity to calculate the chemical polarity (lipophilic or hydrophilic).

The active extract was subsequently blended with Celite in ratio of 1 gram dry crude extract to 1 gram Celite and dried in a vacuum evaporator. The Celite/sample blend was then used for the preparation of a pre-column. Separation was obtained by using a C18 reverse phase column (Puriflash C18-AQ 30 µm F0120 (Interchim)) Water (eluent A)and acetonitrile (MeCN, eluent B) were used starting from A:80%/B: 20% for 8 min and then rising linear to 100% MeCN over 45 min. Full MeCN flow was maintained for another 10 min. The fraction collection was performed peak dependent with the detector collecting peaks appearing in 210 nm and 254 nm wavelength and the Evaporative Light Scattering Detector (ELSD).Collected reaction tubes were combined in logical order to reunite separated peak fractions and the solvents were evaporated. The remaining substances were then resolved in methanol with a concentration of 25 mg/mL and retested on antimicrobial activity by growth inhibition (3.7.2).

4.8.3 Mass spectrometry

The samples were analysed on a high-resolution quadrupole time-of-flight mass spectrometer (QqTOF-ESI-HRMS) from Bruker Daltonics (Bremen, Germany) running with oTOF Control v3.4 and Compass

v1.7. The instrument was equipped with an orthogonal ESI source. Source parameters were adjusted as follows: capillary voltage 4.5 kV; end plate offset 500 V, nebulizer 1.6 bar, dry gas 8 L/min with a dry temperature of 200°C. Samples were screened in the positive-ion mode. The mass spectrometer was coupled to a Dionex UltiMate 3000 HPLC system running under Chromeleon Express (Dionex, Germany). Both instruments were controlled by HyStar v3.2 SR 4. For separation, an Acclaim 120, C8, 3 µm, 120 Å, 2.1 × 150 mm column (Dionex) was used at a flow rate of 0.25 mL/min at 35°C. Eluent A consisted of 0.1% formic acid in distilled water, and eluent B was 0.1% formic acid in MeCN. Amounts of 10 µL of crude extract (50 mg/mL) solutions in MeOH were injected in the column. The following gradient was applied for separation: 5 min at 20% B, from 5 to 55 min a linear gradient from 20% B to 100% B, from 55 to 70 min the column was held at 100% B, from 70 to 71 min the solvent was returned to 20% B, and from 71 to 85 min the column was equilibrated at 20% B.

4.8.4 Nuclear magnetic resonance analysis (NMR)

To investigate the structure of unknown chemical compounds, NMR analysis was used. Therefore, the pure substance serrawettin W2 was submitted to measure proton (^{1}H), carbon (^{13}C), correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC) spectra on a Bruker AV400 spectrometer. Chemical shifts were referenced to the MeOH-d6 solvent residual peaks, δH= 3.310 ppm for 1H and δH=49.000 ppm for C^{13}.

4.9 Strains

The following clinically relevant strains were used to measure antimicrobial activity in a high-throughput set up at our Sanofi-Fraunhofer cooperation group:

Table 1: Organisms used for bioactivity tests

Organism	Strain Number
Candida albicans	in-house strain of Sanofi Frankfurt
Escherichia coli	ATCC25922
Mycobacterium smegmatis	ATCC607
Escherichia coli	D31 K12
Pseudomonas aeruginosa	ATCC27853
Staphylococcus aureus	ATCC25923
Staphylococcus aureus	DSM 799
Saccaromyces cerevisiae	

Material

Table 2: Chemicals

Substance	Supplier
2-Propanol, 70%	Roth
2-Propanol, Rotisolv®	Roth
Acetic acid ethyl ester	Roth
Acetone, 99.5%	Roth
Acetonitrile Rotisolv® UV/IR Grade	Roth
Agar-Agar, BioScience Grade	Roth
Ampicillin Sodium Salt	Roth
Brain Heart Infusion Agar	Roth
Dichloromethane	Sigma-Aldrich
Egg Yolk Emulsion	Roth
Ethanol, Rotipuran	Roth
Ethylenediaminetetraacetic acid	Roth
Gentamycin	Sigma
Formic acid 45%	Roth
Fluconazole	Sigma
Kanamycin sulphate	Roth
LB Broth (Lennox)	Roth
MacConkey Broth	Roth
Mannitol-Salt-Agar	Roth
Mannitol-Egg yolk-Polymyxin	Roth
Methanol Rotisolv®	Roth
SDS Pellets ≥ 99%	Roth
PBS Roti®fair PBS 7.2	Roth
Streptomycin sulphate	Roth
Tryptic Soy Broth	Sigma
TrisPufferan	Roth
Tween 80	Sigma-Aldrich
Water BioScience Grade	Roth
Yeast Glucose Chloramphenicol Agar	Roth

Table 3: Consumables

Material	Supplier
50 mL Cellstar® Polypropylene Tube	Greiner Bio-One
15 mL Cellstar® Polypropylene Tube	Greiner Bio-One
Acclaim® 120 3 µm C8 120 Å, 150 x 2.1 mm	Thermo Fischer
BacTiter-Glo	Promega
Celite 454	ServaElektrophoresis GmbH
Cellstar® 96 Well Suspension Culture, F-bottom, clear	Greiner Bio-One
Cellstar® 96 Well Suspension Culture, F-bottom, white	Greiner Bio-One
Gloves TouchNTuff®	Ansell
Parafilm® M	Roth
Puriflash C18-AQ 30 µM F0120	Interchim
Roti®-Store cryo vials	Roth
Genomic DNA Buffer Kit	Qiagen
Genomic tip 500/G	Qiagen
GeneJet Gel Extraction Kit	Thermo
Mix2Seq Sanger Sequencing Kit	Eurofins

Table 4: Devices

Machine	Model	Company
Air compressor	CPM 160-8-6 W oil free	CompactMaster
Autoclave	5075 ELV	Tuttnauer
Autoclave	3850 EL	Tuttnauer
Balance	ABT 220-5DM	Kern
Balance	Excellence XA 1502 S	Mettler Toledo
Balance	Excellence XA 105 Dual Range	Mettler Toledo
Biosafety cabinet	MSC-Advance	Thermo Scientific
Breeding box	Faunarium	HAGEN Deutschland GmbH & Co
Centrifuge	Mikro 220R	Hettich
Centrifuge	Rotina 420R	Hettich
Dishwasher	Compact Desinfektor G7783 CD Mielabor	Miele
Electrophoresis Power Supply	EV231	Consort
FLASH chromatography	PuriFlash	Interchim
Freezer -20°C	Froster	Kirsch
Freezer -80°C	6343-6345/6383-6385	GFL
Gel Documentation Station	VersaDoc Imaging System 4000 MP	BioRad
Gel Electrophoresis Chamber	Mini-PROTEAN Tetra System	BioRad
Hearing Protectors	Sperian T1	Howard Leight
Hearing Protectors	Optime I	Peltor
Hotplate Stirrer	Hotplate Stirrer Model L-81	Labinco
Hotplate Stirrer	VMS-A	VWR
HPLC	UltiMate 3000	Dionex
Ice Machine	AF 80	Scotsman
Incubator	Multitron	Binder
Lyophilizer / Freeze dryer	RVC 2-33IR	CHRIST
Microscope	DM2500 LED	Leica

Microplate Reader	Eon	Biotek Instruments
Microplate luminometer	LUMIstar OPTIMA	BMG Labtech
Microwave	Grill Hot Air	Sharp
Multichannel Pipette	Rainin Pipet-Lite XLS 2-20 µL	Mettler Toledo
Multichannel Pipette	Rainin Pipet-Lite XLS 20-200 µL	Mettler Toledo
Multichannel Pipette	Reference® 2 10-100 µL	Eppendorf
PCR Cycler	C1000 Thermal Cycler	BioRad
pH-Meter	Seven Multi	Mettler Toledo
Pipette	Rainin Pipet-Lite XLS 0.1-2 µL	Mettler Toledo
Pipette	Rainin Pipet-Lite XLS 2-20 µL	Mettler Toledo
Pipette	Rainin Pipet-Lite XLS 10-100 µL	Mettler Toledo
Pipette	Rainin Pipet-Lite XLS 20-200 µL	Mettler Toledo
Pipette	Rainin Pipet-Lite XLS 100-1000 µL	Mettler Toledo
Pipette	Reference® 2 0.5-10 µL	Eppendorf
Pipette	Reference® 2 10-100 µL	Eppendorf
Pipette	Reference® 2 20-200 µL	Eppendorf
Pipette	Reference® 2 10-1000 µL	Eppendorf
Purified Water System	TKA-GenPure	Thermo
Refrigerator	Super	Kirsch
Evaporator	Rotavapor® R-100	Büchi
Shake Incubator	Multitron II	Infors HAT
Thermoshaker	TS-100 SC-20	bioSan
Ultrasonic Bath	Sonorex	Bandelin
Vortex	VV3	VWR

Table 5: Primer

Name	Sequence	Reference
27F	AGAGTTTGATCMTGGCTCAG	(Lane, 1991)
1492R	ACCTTGTTACGACTT	(Lane, 1991)
ITS1	TCCGTAGGTGAACCTGCGG	(White et al., 1990)
NL4	CTTCCGTCAATTCCTTTAAG	(O'Donnell, 1993)

Results

Aim 1: Isolation, cultivation, and identification of gut microbes

6.1 Beetles and breeding

In a first step, it was tried to catch *N. vespilloides* in their natural habitat (see Material and Methods section). The first beetles were caught in Schiffenberg Forest in mid of May, when temperatures during night time were constantly above 8 °C. During the summer months, catching of *Nicrophorus* species was relatively easy. As soon as the cadaver emitted a strong odour, beetles could successfully be caught even within a few hours. Catching was most successful when traps were baited 3-4 hrs before sunset.

In order to limit the possible impact of a chage from habitat soil one of those artifical soils usually used in literature, *N. vespilloides* was kept plastic boxes filled (see Material and Methods section) filled with soil material from Schiffenberg Forest. The substrate was moistened with tap water whenever it appeared to be dry. Non-breeding beetles were fed with larvae of *Galleria mellonella* twice a week. To induce breeding, a dead mouse was provided. As soon as a couple of beetles had successfully conquered and defended the carcass, the beetles started preparing it by shaving off the fur. After this procedure, the carcass was buried in the soil, and beetles started breeding. Within a few weeks, mature L3 larvae could be observed. Unfortunately, no adults emerged from the pupation chamber. Therefore, any attempts of breeding *N. vespilloides* under those artifical laboratory conditions were stopped, and the beetles were either caught on demand, or parasite-free animals were provided from the laboratory of Dr. H. Vogel (MPI, Jena) and dissected directly.

6.2 Dissection

In order to obtain gut microbes, cooled beetles were directly dissected under a binocular microscope using a micro forceps. Surface contaminants of the beetle were killed and removed as described in **chapter 3.2**. The aim of this dissection was to remove the gut without disrupting and spilling of its content.

Due to its life cycle, *Nicrophorus* spp. evolved a very hard cuticle, especially around the head. To open up the body cavity, elytra were removed, and the abdomen was carefully accessed. Every abdominal segment was thoroughly removed, and the last abdominal part was handled with utmost care to prevent perforation of the beetle's rectum. In a next step, the pronotum of the beetle was removed carefully by using a micro scissors and a micro forceps. The anterior part of the gut is arranged in a straight line, but the hindgut is coiled on top of the midgut. Only the last part is straightened to the rectum, which serves a reservoir for anal secretions (**Figure 7**).

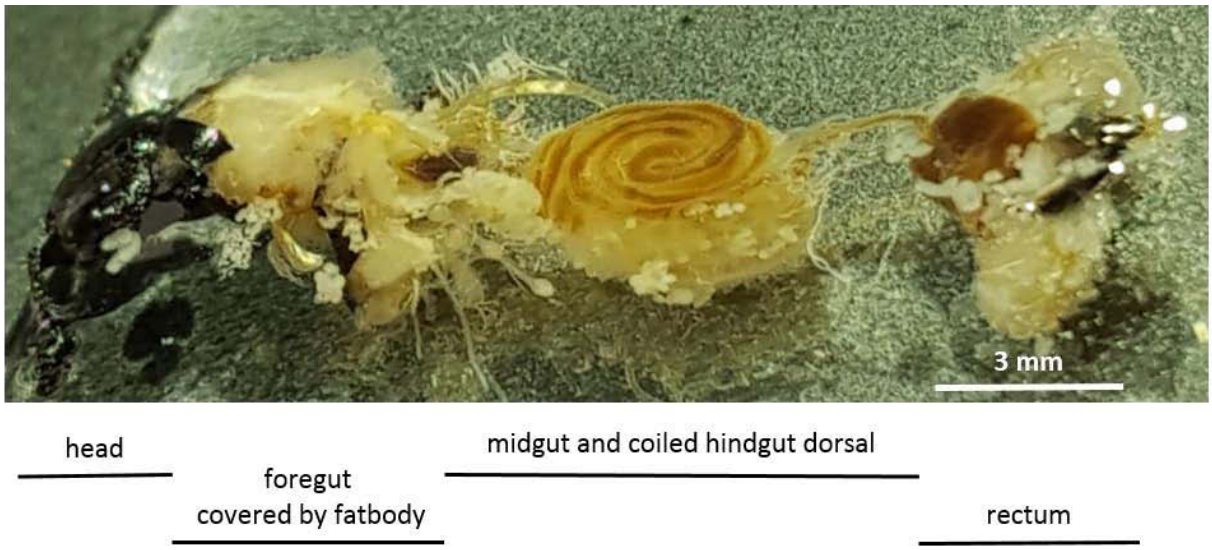


Figure 7: Dissection of *N. vespilloides.*
The dissected body of the beetle unveils the orientation of the gut. The head is still partly covered by the hard cuticle, followed by a fat body covering the foregut. The coiled hindgut is clearly visible, on top of the voluminous midgut segment. The filled, dark brown coloured rectum is completely embedded in fat body.

Afterwards the gut was separated in its three compartments. In most cases, the foregut and midgut had to remain together because of the shortness of the former. In contrast, hindgut and rectum could easily be separated from each other. The resulting three segments were physically opened by crushing (see Material and Methods section) to get access to the gut lumen with its crypts. A self-made piston (from a 1000 µL-pipette tip) was found to be superior to the use of a tissue-lyser system for breaking up these gut segments

6.3 Cultivation of the gut microbiome

6.3.1 Cultivation of bacteria

A decisive part of this study was aimed at cultivating the beetle's gut bacteria and eukaryotic microbes. First of all, a number of suitable complex and standard selective media for cultivation of gut microbiotia had to be chosen. Therefore, it was important to consider the composition of the beetles' natural diet. Consequently, complex media rich in proteins and amino acids were used to cultivate the gut. Brain Heart Infusion (BHI) was chosen as a rich and sufficient source of those components. The bacteria from laboratory-hatched beetles were cultivated after an overnight enrichment step in BHI media. The inocula were afterwards serially diluted from 10^3 to 10^6 and spread on BHI agar. Bacterial colonies were picked and streaked on fresh BHI agar plates for multiple times to obtain pure cultures for 16*S* amplification and cryo storage.

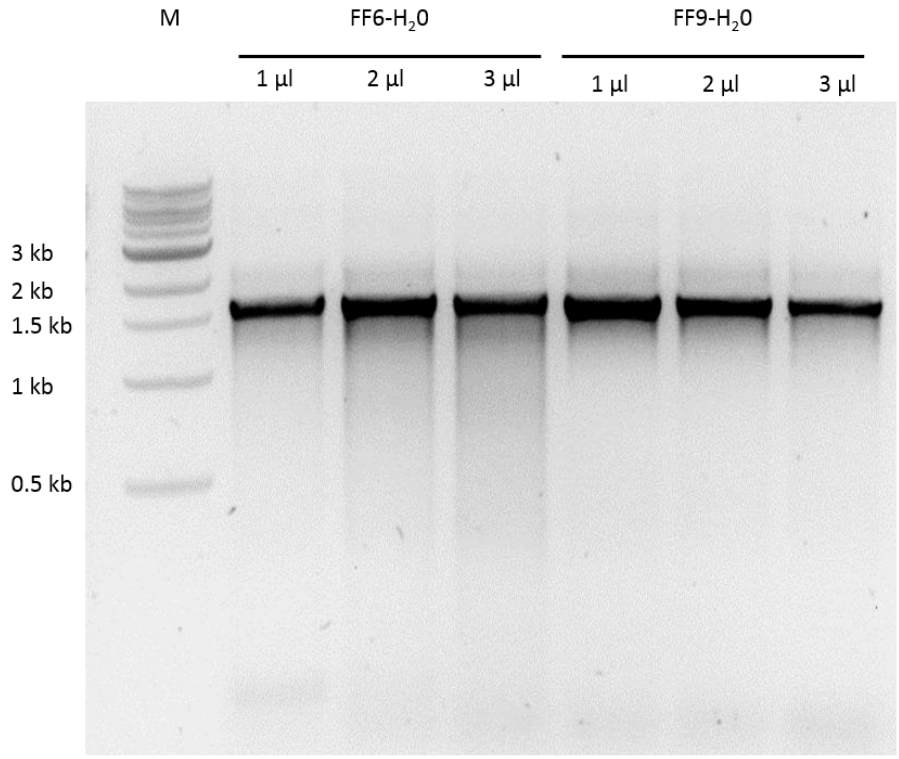


Figure 8: Example of 16S rRNA amplification.
Colony PCR was performed with the denaturised cell suspension and was used as the template for the PCR with different amounts (1/2/3 µL) to amplify the 16S ribosomal gene. The resulting product is 1465 bp long as the 1.5 kb band of the marker also indicates. The shown samples are isolated from female foregut sample number 6 and 9, cultivated on H2O agar (FF6-H2O and FF9-H2O).

This enrichment step might have supported the growth of generalists. Consequently, this procedure was not considered for the final, optimised isolation process. In contrast, direct plating of the diluted gut fragment suspension on solid media resulted in sufficient microbial growth and an improved colony picking. To limit excessive growth of fast-growing and swarming bacteria, agar of low nutrient concentration and even water agar were used. Growth sufficient for selective colony picking was

reached after a period of time of up to 3 weeks to. Ampicillin and kanamycin were used to inhibit the growth of sensitive bacteria and to select for resistant strains. Both antibiotics were chosen because of their different mode of action.

In this study, 320 bacterial samples were isolated from the gut of *N. vespilloides* and sequenced after 16*S* gene amplification. The Basic Local Alignment Search Tool for nucleotides (BLASTn) was subsequently used for identification. The results are shown in **Figure 9**. Bacteria were identified to the genus level based on their 16*S* gene sequences. The species diversity could not be taken into account because of the insufficient species identification based on the 16*S* gene.

Microorganisms were cultivated mainly on BHI agar but also on TSB as well as on different selective media such as mannit yolk polymyxin agar (MYP), mannit salt agar (MSA), water agar (H_2O), yeast extract glucose chloramphenicol agar (YGC) and peptone glucose starch agar (PGS)

.

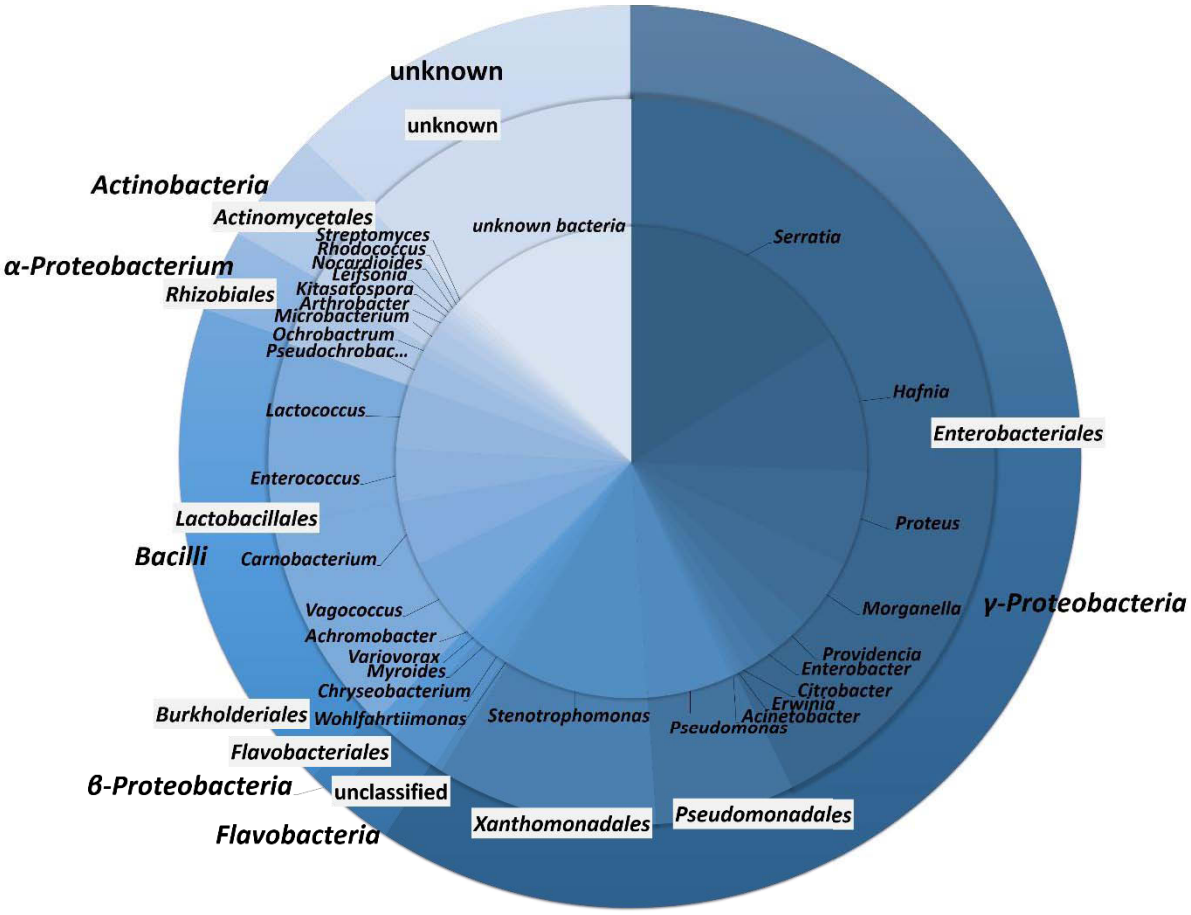


Figure 9: Bacterial diversity of the cultivable gut microbiome.
From outside to inside: class→order→genus
The field sizes are based on abundance. Identification was achieved by 16*S* gene amplification followed by BLASTn search.

The most abundant bacteria isolated were of the class of *Gammaproteobacteria* with a total of 59.1%. The family of *Enterobacteriales* was cultivated with ~ 43% prevalence of all bacterial families, followed by ~ 10% *Xanthomonadales* and ~ 6% *Pseudomonadales.* The most abundant genus was *Serratia* with a total of ~ 43% among all isolates belonging to the *Enterobacteriales*, followed by the genera *Hafnia*

(~ 22%) and *Proteus* (~ 15%). Genera of regular but lower abundance comprise *Morganella* (~ 11%) and *Providencia* (~ 7%), followed by *Enterobacter*, *Citrobacter* and *Erwinia*.

Beside the order of *Enterobacteriales*, *Xanthomonadales* and *Pseudomonadales* were also identified. They were represented by the genera of *Pseudomonas* (*Pseudomonadaceae*) and *Stenotrophomonas* (*Xanthomonadaceae*). Next to the *Gammaproteobacteria*, the class of *Bacilli* (17.2%) was the second most abundant represented by the order *Lactobacillales*. The genera *Carnobacterium*, *Vagococcus* and *Achromobacter* as well as *Lactococcus* and *Enterococcus* were the most abundant. The third largest group of isolated bacteria is previously not further specified with a total of 12.8% percent. Next to these identified two major classes, *Alphaproteobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Flavobacteria* were also isolated and cultivated. In total, 25 different genera were isolated, next to some undefined species of *Microbacterium*, *Lactococcus and Enterobacteriaceae*. The full BLASTn identification list can be found in the appendix (**Table S1**).

During the process of undirected bacterial isolation on complex media, strong swarming occurred irregularly. In those cases a reduction of the nutrient yield limited the swarming. In general, 100% BHI agar was used; however, if swarming occurred, especially with *Proteus mirabilis*, the nutrient concentration was reduced to 50%.

It should be pointed out that the cultivation of the microbiome aimed at all bacteria, not only at those that have already been scientifically described. However, the isolation of unspecified bacteria was achieved extremely rarely. Difficulties in the isolation of those bacteria might have arisen from numerous reasons. Strain 3MH1, isolated from the hindgut of a male beetle, was grown from a diluted gut suspension, which has been plated out on TSB agar. After colony picking, PCR amplification and Sanger sequencing, the BLASTn analysis revealed an identity coverage of 95.3% with its closest ancestor *Wohlfahrtiimonas larvae*. However, a major problem arose after several successful passages on solid agar media and cryo conservation. The strain 3MH1 failed to grow from cryo stocks as well as from the agar plates so that a further characterization was impossible. A change of growth conditions was a first starting point to resolve this issue. The strain was grown at different cultivation temperatures, i. e., 4°C, 15°C 21°C, 26°, 30°C and 37°C. Growth was regularly checked over a period of 4 weeks, but no colonies could be detected, anymore. Thereafter, the strain was grown under microaerophilic and anaerobic conditions. Even the use of an anaerobic jar did not lead to success. Supplementation of the growth medium with iron(II)sulfate ($FeSO_4$), which is commonly recommended as a strategy for isolation and cultivation of rare bacteria with siderophore activity, showed no effect either.

Cultivation on non-selective media in an anaerobic jar revealed the presence of the genera. *Carnobacterium* (*Latobacillales*, *Carnobacteriaceae*), and *Enterobacter* (*Enterobacteriales*, *Enterobacteriaceae*). Both genera are facultatively anaerobic and were also found under aerobic

conditions. Overall, time and effort required for anaerobic cultivation were comparatively high; and the low taxonomic diversity of the isolates obtained did not justify any future application of this method.

6.3.2 Cultivation of fungi

For cultivation of eukaryotic microorganisms, a selective medium, yeast extract glucose chloramphenicol agar (YGC), was used successfully. Chloramphenicol is a broad-spectrum antibiotic, which is supplemented here to prevent bacterial growth, thus promoting the selection of yeasts and fungi. The cultivation itself was performed in the same manner as for bacteria, i. e., by direct plating of 10^1 diluted gut suspensions.

Figure 10 summarizes the results of the eukaryotic cultivation approach. With 68% of all isolates, *Yarrowia* was the most frequently isolated genus of all eukaryotic isolates. The genus *Yarrowia* (*Saccharomycetales*, *Dipodascaceae*) belongs to the true yeasts. BLASTn search either revealed *Yarrowia lipolytica* (28%) or *Yarrowia sp.* (40%). Isolates identified as *Yarrowia sp.* had a BLAST coverage of 98% of identity for the amplified ITS regions, indicating a genetic difference compared to the references. The genus *Candida* (*Saccharomycetales*, *Saccharomycetaceae*) was the second most common (8%) fungal isolate.

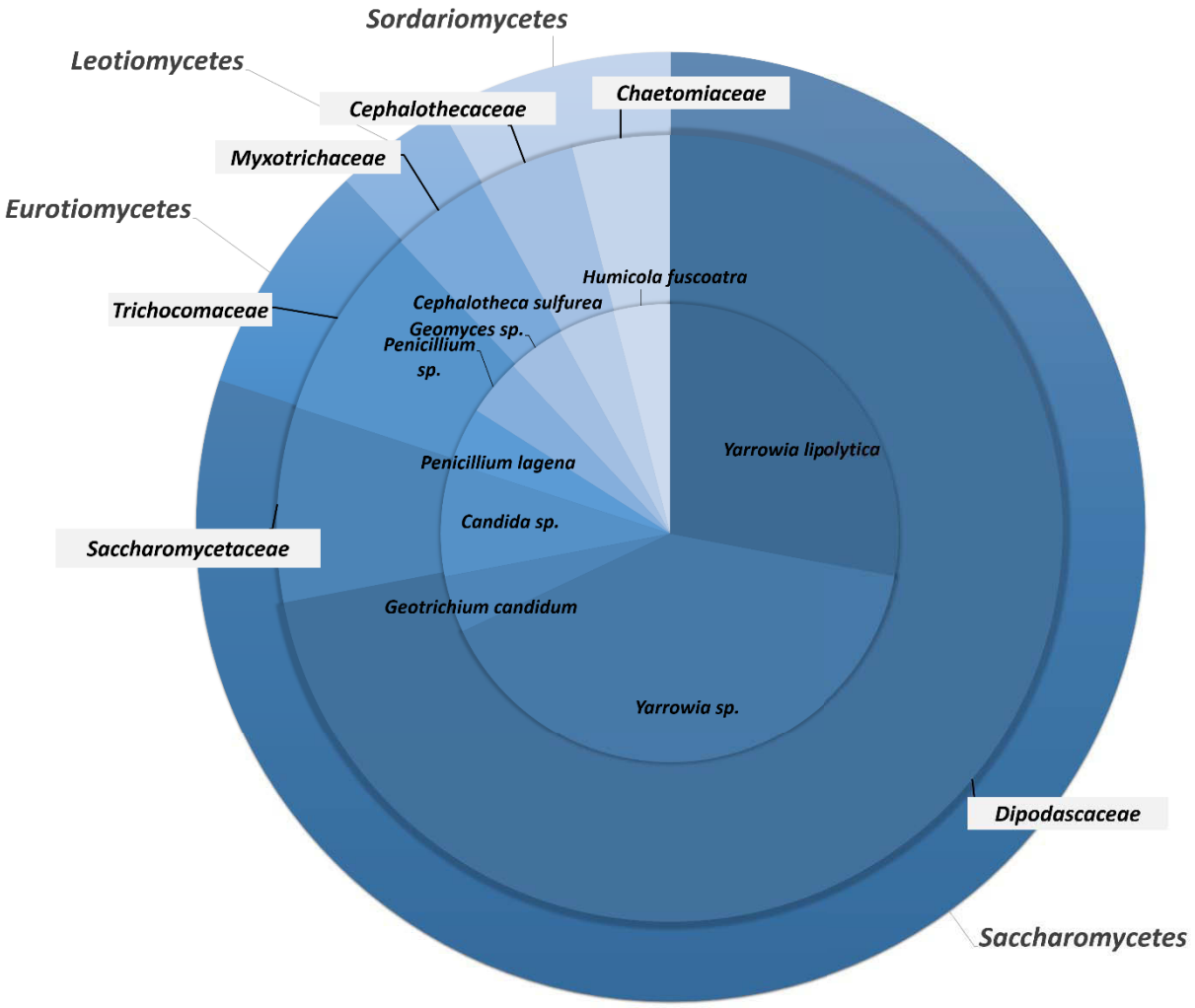


Figure 10: Fungal diversity of the cultivable gut microbiome.
Outside to inside: class→order→genus
The most abundant isolates were *Yarrowia lipolytica* and *Yarrowia sp.* of the family *Dipodascaceae* and *Candida sp*. of the family *Saccharomycetaceae.*

Cultivation of *Yarrowia* species revealed obvious differences in the morphology of the isolates. At least four different colony shapes could be distinguished when isolates were growing on the same YGC agar plate (**Figure 11**).

Full genome sequencing has been performed for *Yarrowia sp*. isolates by our collaboration partners from the Max Planck Institute for Chemical Ecology and Dr. Karina Brinkrolf. The results suggest different subspecies but this cannot be linked to the morphological differences, so far. Most notably, *Yarrowia* was the only fungal genus isolated from the rectum of the beetle, and it seems to be endemic to this gut compartment.

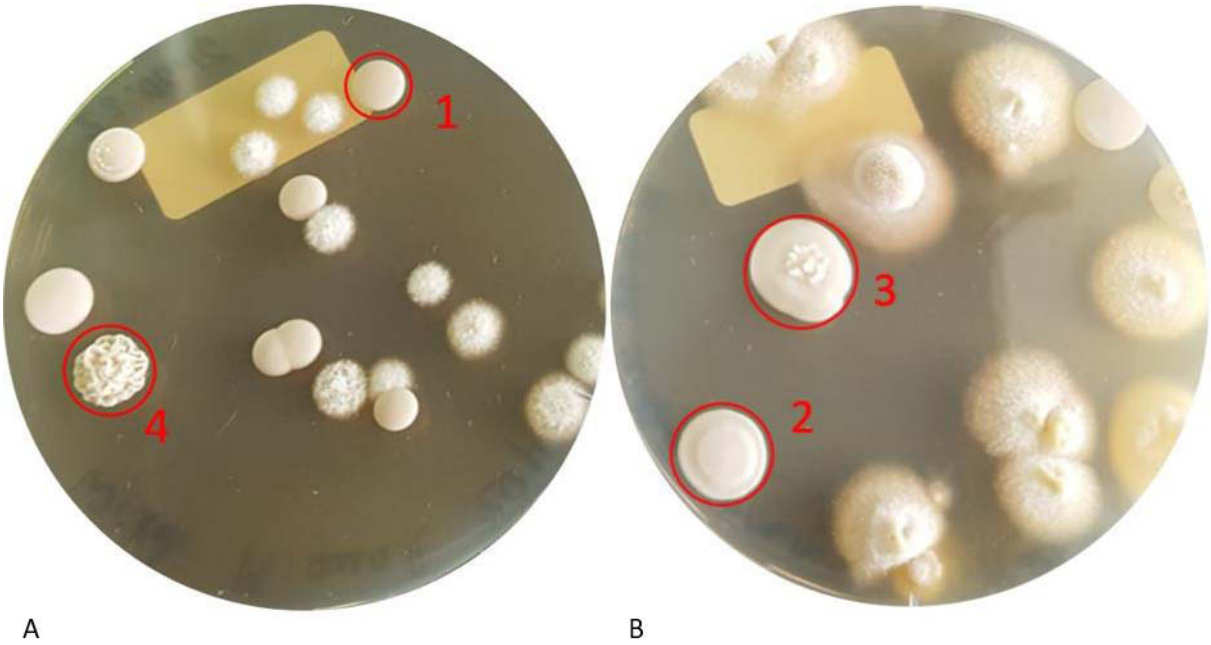


Figure 11: Differences in growth morphology of *Yarrowia* isolates.
The isolates growing on YGC agar plates are yeasts of the genus *Yarrowia* and fungi of the genus *Candida*. Under the same growth conditions, the *Yarrowia* isolates exhibit different morphologies. .Red circles indicate different growth shapes of *Yarrowia* colonies. Smooth (**1**) can be found as well as concave (**2**) and spiky (**3**) colonies in different orientation. The most divergent shape, however, is best described as a spaghetti-shaped colony (**4**).

Besides the genus *Yarrowia*, the genera *Penicillium* (*Eurotiales*, *Aspergillaceae*), *Geomyces* (Incertae sedis, *Pseudeurotiaceae*) as well as *Cephalotheca* (*Sordariales*, *Cephalothecaceae*) and *Humicola* (*Sordariales*, *Chaetomiaceae*) were cultivated.

Aim 2: Fermentation, chemical extraction, and antimicrobial screening

6.3 Testing for antimicrobial activity

6.3.1 Pre-screening with crude extracts

After the cultivation of 320 bacterial isolates from the gut of both wild and laboratory-reared *N. vespilloides* beetles, a selection of strains based on 16*S* gene similarity was made. Isolation and cultivation of the microbial samples was based on morphological differences of the colonies obtained. Therefore, the genetic differences indicated by 16*S* analysis were used for the following screening of antimicrobial activity. Based on that, 113 bacterial strains and 6 *Yarrowia* isolates were selected. This de-replication process is limited by the genetic differences in the bacterial 16*S* gene but was necessary for the reduction of the number of isolates. Because of this selection, at least one representative of each species was chosen for fermentation. All of the 119 microbial isolates were grown in liquid culture in conical flasks (Erlenmeyer-Kolben). Afterwards, the cultures were freeze-dried and extracted. Liquid cultures were sampled after one, six and nine days, respectively, because the time-point of induction of antimicrobial compound production is not predictable. Secondary metabolite production can be induced by numerous metabolic events. It can result, for example, from nutrient limitation, from growth during the stationary phase.etc. In this study, a strong increase in antibiotic activity was detected between the 1st and 9th day of cultivation. The freeze dried material was subsequently extracted with EtOAc and MeOH, as described in the Material and Methods section. Only five crude EtOAc extracts displayed sufficient antimicrobial activity, whereas the number of bioactive MeOH extracts was significantly higher (231 extracts).

The primary screening was performed with a set of relevant microbial organisms, including Gram-negative pathogens such as wild-type *Escherichia coli*, the efflux pump mutant *E. coli* ΔTolC, and *Pseudomonas aeruginosa.* Gram-positive bacteria such as *Staphylococcus aureus* and *Mycobacterium smegmatis* were also included as well as the eukaryotic human pathogen *Candida albicans*. However, it should be kept in mind that *M. smegmatis*[1] is a highly sensitive, organism. Consequently, the high inhibition rates recorded from testing of crude extracts have to be scrutinised in order to avoid false positive hits. In a later stage of screening, *E. coli* ΔTolC had to be excluded from the spectrum of test organisms because of inconstant growth, which corresponded to false positive inhibitions.

By screening the crude extracts for antimicrobial activity, a huge number of active isolates was identified (**Figure 12**). The highest number of antimicrobially active extracts originated from the group of undetermined isolates – remarkably, a total of 26% of all strains was found in this group.

[1] *M. smegmatis* is used as a safety level 1 screening strain for tuberculosis.

Surprisingly, most of the bioactive isolates were found in the orders of *Pseudomonadales* (19%), *Lactobacillales* (17%), and *Enterobacteriales* (17%). In contrast, the orders *Actinomycetales* (10%), *Flavobacteriales* (2%) and *Burkholderiales* (2%), which are most well-known as classical sources of antibiotics, were less represented. Here, it has to be emphasised that only a small number of isolates obtained from *Nicrophorus* guts belonged to these orders.

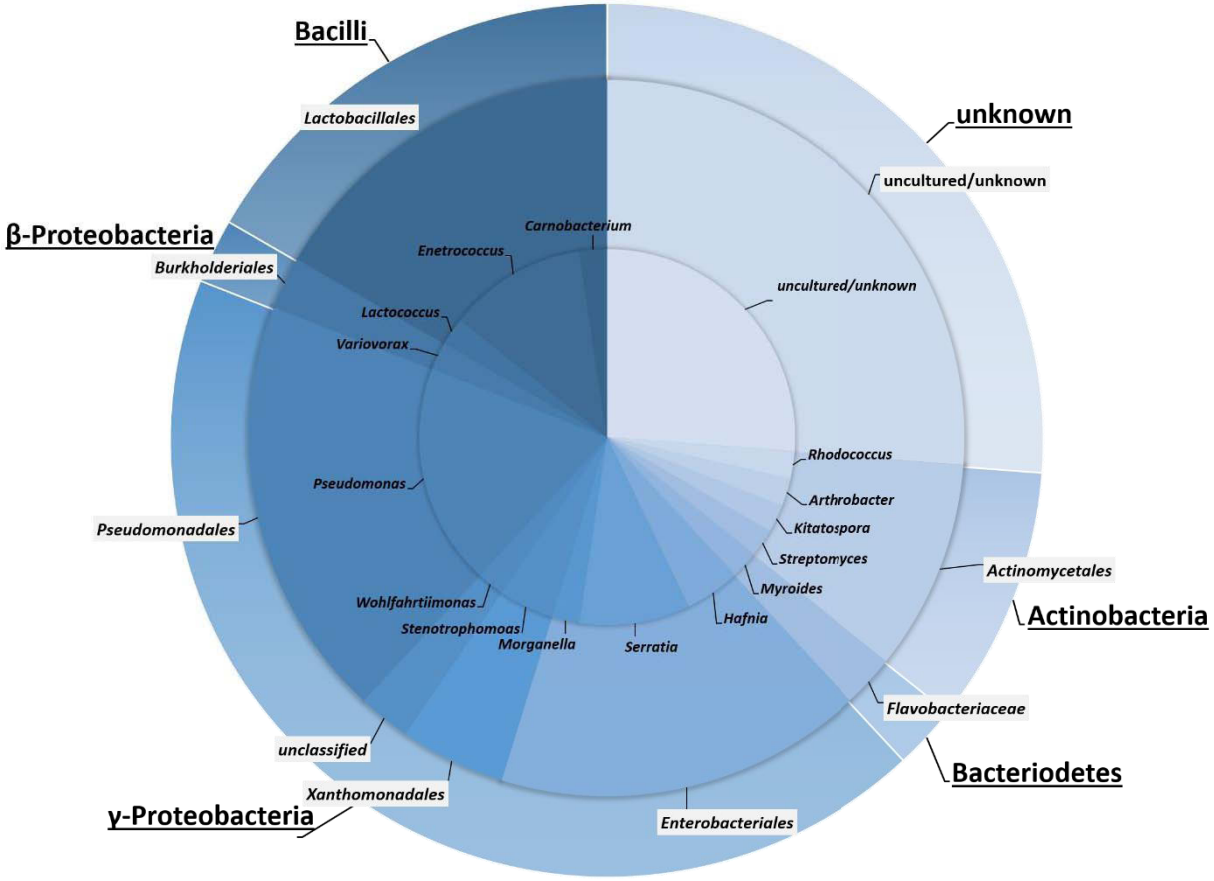


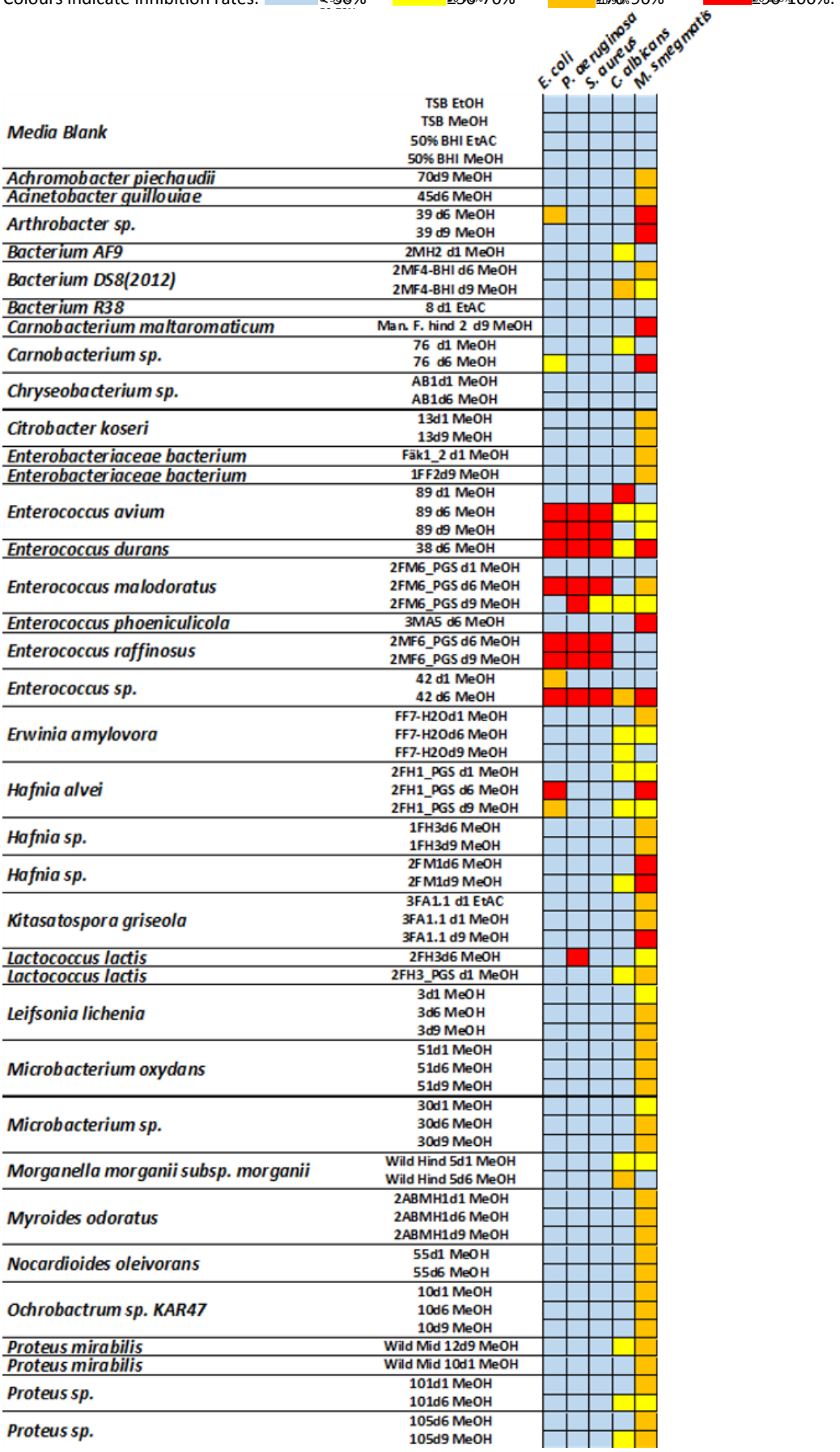
Figure 12: Taxonomic diversity of bacteria producing bioactive crude extrats.
Outside to inside: class→order→genus
The diagram combines all observed antimicrobial activities from the 113 bacterial isolates tested. More than 26% of all biologically active extracts were obtained from undetermined bacteria. Strains of the genus *Pseudomonas* were the most abundant producers of bioactivity, compared to the other genera.

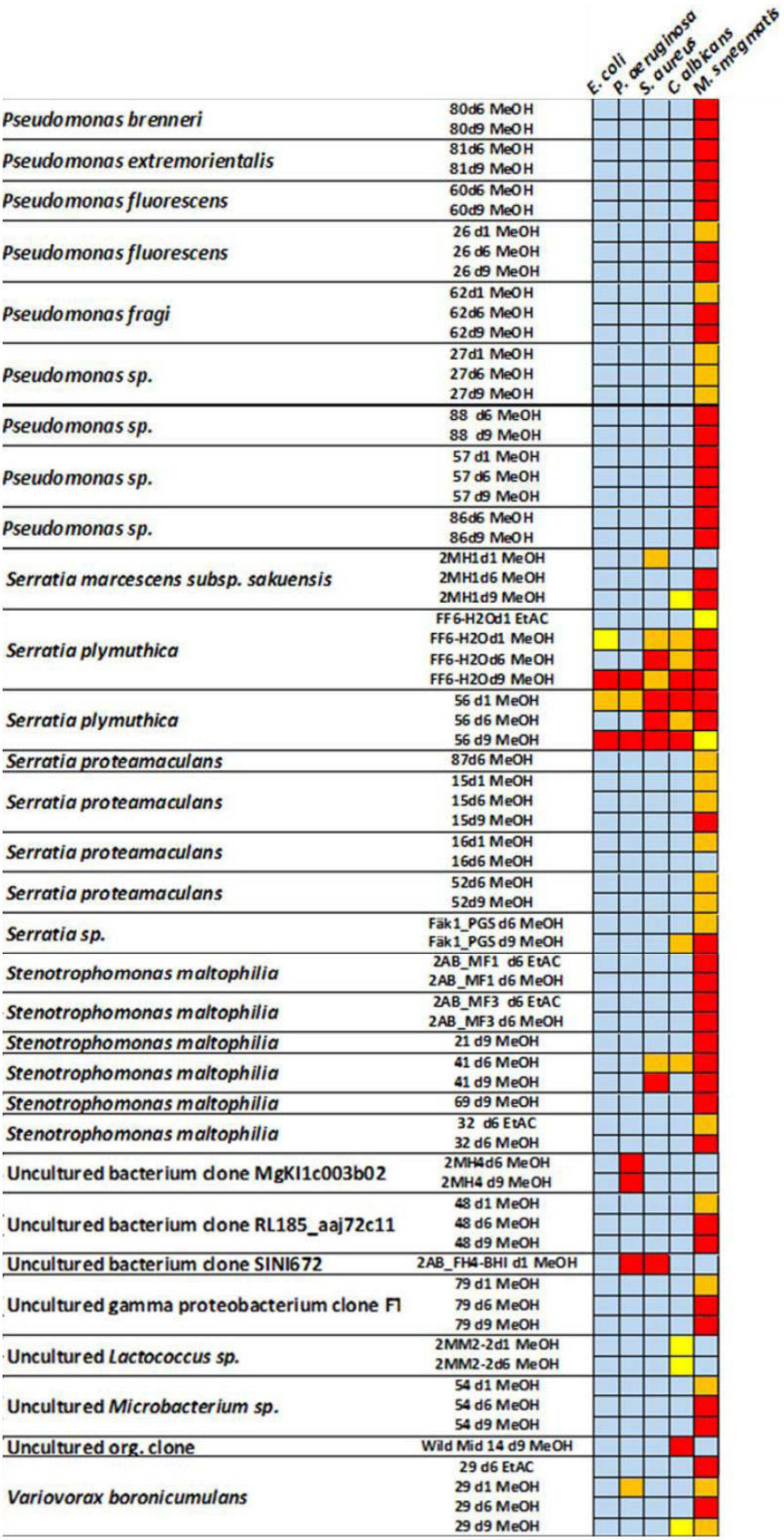
Screening of crude extracts resulted in a huge number of antimicrobial activities (**Table 6**) against the pathogens tested. For instance, the *Serratia* isolate FF6-H_20 or the *Enterococcus* isolate 42, exhibited broad-spectrum activity against all test organisms. In total, 71% of all MeOH extracts showed ≥ 70% inhibition of *M. smegmatis.* In contrast, selective inhibition of *C. albicans* has only been observed comparatevely rarely. One of those examples is the unspecified isolate Wild Mid 14, obtained from the midgut of a wild living beetle.

The least inhibition rates were recorded against Gram-negative bacteria with only 11.5% of all extracts displaying sufficient inhibition rates of ≥ 70%. Crude extracts that are active against Gram-negatives

are regarded most interesting. This is due to the fact that the pharmacutical market is devoid of novelties in this field of application. Inhibition of *E. coli* and *P. aeruginosa* is therefore of high value for the developement of modern, selective antibiotics. As examples, the MeOH extracts of isolate 2FH1_PGS (genus *Hafnia*) and the so far undetermined bacterium 2MH4 can be listed. Those extracts were capable of effectively inhibiting either *E. coli* or *P. aueruginosa*. Remarkably, the genera *Enterococcus* and *Serratia* (*Enterobacteriales*) produced extracts that exhibited a pronounced anti-Gram-negative activity.

Table 6: Heat map of selected antimicrobial effects produced by the gut bacteria.
Colours indicate inhibition rates: <50% ≥50-70% ≥70-90% ≥90-100%.




E. coli
P. aeruginosa
S. aureus
C. albicans
M. smegmatis
Pseudomonas brenneri
80d6 MeOH
80d9 MeOH
Pseudomonas extremorientalis
81d6 MeOH
81d9 MeOH
Pseudomonas fluorescens
60d6 MeOH
60d9 MeOH
Pseudomonas fluorescens
26 d1 MeOH
26 d6 MeOH
26 d9 MeOH
Pseudomonas fragi
62d1 MeOH
62d6 MeOH
62d9 MeOH
Pseudomonas sp.
27d1 MeOH
27d6 MeOH
27d9 MeOH
Pseudomonas sp.
88 d6 MeOH
88 d9 MeOH
Pseudomonas sp.
57 d1 MeOH
57 d6 MeOH
57 d9 MeOH
Pseudomonas sp.
86d6 MeOH
86d9 MeOH
Serratia marcescens subsp. sakuensis
2MH1d1 MeOH
2MH1d6 MeOH
2MH1d9 MeOH
Serratia plymuthica
FF6-H2Od1 EtAC
FF6-H2Od1 MeOH
FF6-H2Od6 MeOH
FF6-H2Od9 MeOH
Serratia plymuthica
56 d1 MeOH
56 d6 MeOH
56 d9 MeOH
Serratia proteamaculans
87d6 MeOH
Serratia proteamaculans
15d1 MeOH
15d6 MeOH
15d9 MeOH
Serratia proteamaculans
16d1 MeOH
16d6 MeOH
Serratia proteamaculans
52d6 MeOH
52d9 MeOH
Serratia sp.
Fäk1_PGS d6 MeOH
Fäk1_PGS d9 MeOH
Stenotrophomonas maltophilia
2AB_MF1 d6 EtAC
2AB_MF1 d6 MeOH
Stenotrophomonas maltophilia
2AB_MF3 d6 EtAC
2AB_MF3 d6 MeOH
Stenotrophomonas maltophilia
21 d9 MeOH
Stenotrophomonas maltophilia
41 d6 MeOH
41 d9 MeOH
Stenotrophomonas maltophilia
69 d9 MeOH
Stenotrophomonas maltophilia
32 d6 EtAC
32 d6 MeOH
Uncultured bacterium clone MgKI1c003b02
2MH4d6 MeOH
2MH4 d9 MeOH
Uncultured bacterium clone RL185_aaj72c11
48 d1 MeOH
48 d6 MeOH
48 d9 MeOH
Uncultured bacterium clone SINI672
2AB_FH4-BHI d1 MeOH
Uncultured gamma proteobacterium clone F1
79 d1 MeOH
79 d6 MeOH
79 d9 MeOH
Uncultured Lactococcus sp.
2MM2-2d1 MeOH
2MM2-2d6 MeOH
Uncultured Microbacterium sp.
54 d1 MeOH
54 d6 MeOH
54 d9 MeOH
Uncultured org. clone
Wild Mid 14 d9 MeOH
Variovorax boronicumulans
29 d6 EtAC
29 d1 MeOH
29 d6 MeOH
29 d9 MeOH

Isolated bacteria were not analysed down to their subspecies diversity, but the different antimicrobial activities between isolates of the same species clearly indicate a chemical diversity. This suggests a subspecies diversity which cannot be uncovered by 16*S* gene analysis.

Therefore, a phylogenetic analysis based on partial 16*S* sequences was used in combination with the spectrum of antimicrobial activity to investigate the isolates of the genus *Serratia* (order: *Enterobacteriales*).

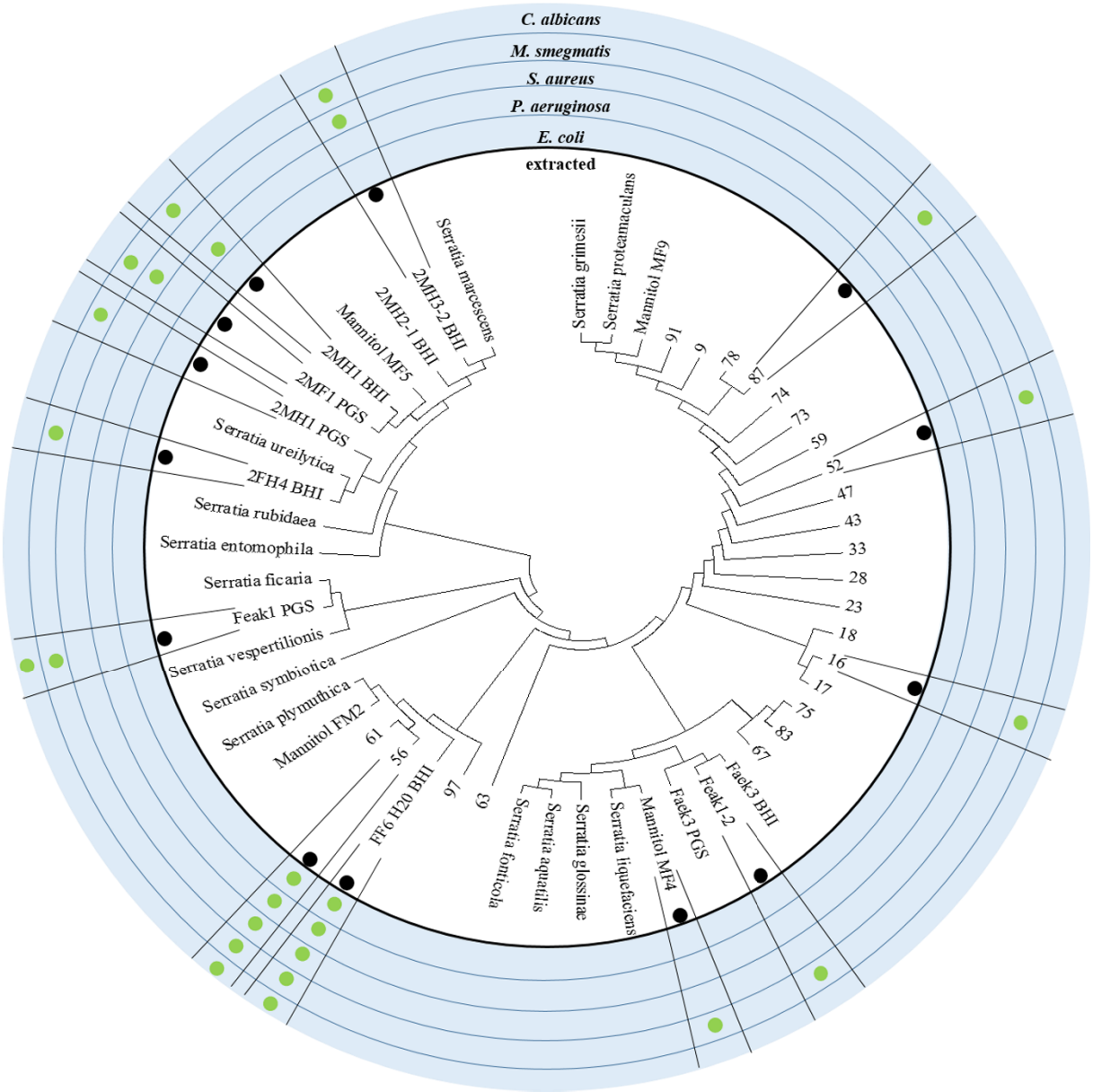


Figure 13 displays the phylogeny of all isolated bacteria identified as members of this genus and the antimicrobial activity of the respective strains. For the phylogenetic tree, all *Serratia* 16*S* forward amplificates were aligned to *Serratia* 16*S* references downloaded from the National Center for Biotechnology Information (NCBI). The reference species include *S. grimesii*, *S. proteamaculans*, *S. ficaria*, *S. vespertiliones*, *S. entomophilia*, *S. symbiotica*, *S. plymuthica*, *S. liquefaciens*, *S. glossinae*, *S. fonticola*, *S. aquatilis*, *S. rubidaea*, *S. ureilytica*, and *S. marcescens*. The alignment was then trimmed

to 650 bp length for equalization. By computing a pairwise distance analysis with MEGA 7, a maximum-likelihood phylogenetic tree was generated. The phylogeny joint with the antimicrobial activity could indicate differences, also within closely related isolates. Comparing, for example, the related isolates 2MH1 PGS and 2MH3-2 BHI in terms of their antimicrobial activity displayed obvious differences. The extracts of both isolates were able to inhibit *M. smegmatis* but only isolate 2MH3-2 BHI was also active against *S. aureus*. Besides this, the isolates 56 and FF6-H2O, both clustering to the reference *S. plymuthica*, exhibited a broad antimicrobial activity against all tested pathogens (see also **Table 6**). Moreover, antifungal activity, represented by the inhibition of *C. albicans*, was rarely observed among the genus *Serratia*. Remarkably, Faek1 PGS was the only strain that was found to inhibit *C. albicans*.

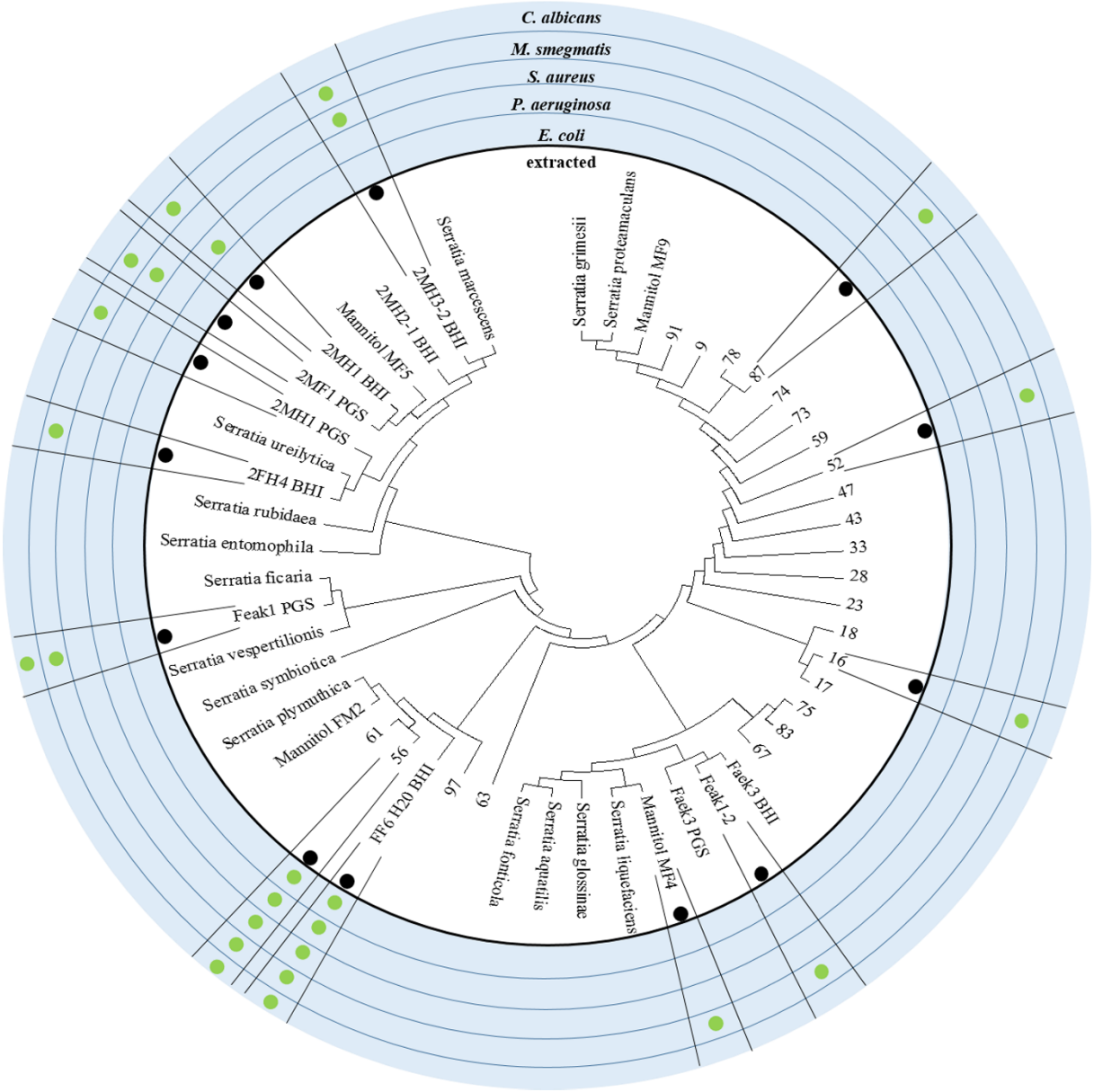


Figure 13: Phylogeny of the *Serratia* isolates and their antibiotic active crude extracts.
● strains tested ● antimicrobial activity
The phylogenetic tree in combination with the antibiotic bioactivity indicates the chemical diversity of closely related strains. The isolates FF6 H_2O and 56 seem to be phylogenetically closely related, as also suggests by bioactivity data. Nevertheless,

closely related *Serratia* isolates, e.g., 2MH3-2 BHI and 2MH1 PGS, both related to *S. marcescens*, differ in their activity against *S. aureus*.

6.3.2 HPLC separation of the crude extracts

To investigate the antimicrobial effects of a crude extract, it is necessary to effectively separate the substances by HPLC. After that, another bioactivity test is required to identify the active fractions and to avoid possible losses of antibiotic activity. A total of 44 bioactive crude MeOH extracts was selected for further investigation. The fractions were collected within 96 well plates and again tested for antimicrobial activity after the solvent mixture of MeCN and H_2O was removed. Subsequnetly, each residue was resuspended with an incoculum of the appropriate test organism. The retesting was carried out in-house using *E. coli* and the yeast *Saccaromyces cerevisiae*, but it revealed no positive results. In addition, all HPLC fractions collected were also tested by the Sanofi-Fraunhofer (Dr. Benedikt Leis), who confirmed these negative results. Surprisingly, only fresh crude extracts displayed antimicrobial activity. This indicated that long-term storage in MeOH at 4°C can lead to the loss of activity. Thus, a complete re-fermentation and extraction of all 44 selected isolates was inevitable. This time, the solvent MeOH was evaporated before storage. Extracts of the numbers 1 to 9 (**Table 7**) were tested against *E. coli*, numbers 10 to 13 were tested against *P. aeruginosa*. Numbers 14 to 18 were tested for antifungal activity against *C. albicans*, and numbers 19 to 21 had to be tested against *S. aureus*. All other extracts were tested against *M. smegmatis*. Afterwards, the crude extracts of the listed strains were separated by HPLC as previously described, and fractions were tested for antimicrobial activity.

Table 7: Selected antibiotic effects by percentage of inhibition from methanol crude extracts.
Dark green: ≥85% inhibition light green: 71-85% inhibition yellow: 50-70% inhibition

	Closest genetic relative	internal strain name	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*M. smegmatis*
1	*Serratia plymuthica*	FF6-H_2O d9	100	100	72	98	100
2	*Enterococcus durans*	38 d6	99	97	91	0	0
3	*Arthrobacter sp.*	39 d6	80	25	0	0	100
4	*Enterococcus sp.*	42 d6	100	100	100	0	0
5	*Carnobacterium sp.*	76 d6	54	31	0	0	0
6	*Enterococcus avium*	89 d9 MeOH	101	101	100	0	0
7	*Hafnia alvei*	2FH1_PGS d6	93	0	0	0	0
8	*Enterococcus malodoratus*	2FM6_PGS d6	100	101	100	0	0
9	*Enterococcus raffinosus*	2MF6_PGS d6	99	98	101	0	0
10	Uncultured bacterium	2AB_FH4 _BHI d1	0	99	97	0	0
11	*Lactococcus lactis*	2FH3 d6	13	100	8	0	55
12	Uncultured bacterium clone	2MH4 d9	3	100	0	0	0
13	*Variovorax boronicumulans*	29 d1	1	87	0	0	85
14	*Hafnia sp.*	2FM1d9	24	0	0	54	95
15	*Morganella morganii*	Wild Hind 5 d6	13	0	0	70	20
16	*Pseudomonas sp.*	20 d1	9	0	51	73	83
17	Uncultured organism clone	Wild Mid 14 d9	1	0	0	94	0
18	Bacterium DS8(2012)	2MF4-BHI d9	0	0	0	73	64
19	*Serratia marcescens*	2MH3-2 d9	11	0	86	0	100
20	*Serratia marcescens*	2MH1 d1	15	0	81	0	49
21	*Serratia marcescens*	2MH1_PGS d6	2	0	68	0	0
22	*Serratia proteamaculans*	15 d9	15	0	0	0	92
23	*Rhodococcus qingshengii*	44 d6	12	12	0	0	93
24	*Pseudomonas fluorescens*	60 d9	19	0	0	0	97
25	*Pseudomonas fragi*	62 d9	23	0	0	21	97
26	*Pseudomonas sp.*	66 d9	16	0	0	0	96
27	*Pseudomonas brenneri*	80 d9	10	0	0	0	97
28	*Pseudomonas extremorientalis*	81 d9	9	0	0	0	100
29	*Pseudomonas sp.*	86 d9	4	2	0	0	100
30	*Myroides odoratus strain*	2ABMH1 d9	0	0	0	0	88
31	*Pseudomonas sp.*	20 d9	11	0	0	0	98
32	Uncultured bacterium clone	48 d1	0	0	0	0	79
33	Uncultured bacterium clone	50 d9	6	0	0	23	99
34	Uncultured *Microbacterium sp*. clone	54 d6	10	0	0	0	97
35	*Stenotrophomonas humi*	71 d9	0	0	0	0	90
36	Uncultured gamma *proteobacterium* clone	79 d6	0	0	0	0	99
37	Uncultured bacterium clone	2ABMF2 d6	0	0	0	0	91
38	*Wohlfahrtiimonas larvae*	3FM1 d1	5	0	0	0	99
39	*Stenotrophomonas pavanii*	3MA3 d9	3	0	0	12	100
40	*Streptomyces herbaricolor*	3FA1 d9	5	0	0	0	100
41	*Enterococcus phoeniculicola*	3MA5 d6	7	0	0	0	95
42	*Kitasatospora griseola*	3FA1.1 d9	7	6	0	0	100
43	Swine effluent bacterium	3MH1 d9	7	0	0	0	100
44	*Pseudomonas sp.*	57 d1	0	0	10	0	100

Because the HPLC separation and the combined collection of the separated substances were performed in a time-dependant manner, it is possible to calculate the retention time of antimicrobially active fractions. This enables to link the HPLC separation to the LC-MS analysis of the extract for further investigations. By a simple well count and a multiplication of the collection time for each well (50 s), the approximate retention time of the bioactive fraction can be determined.

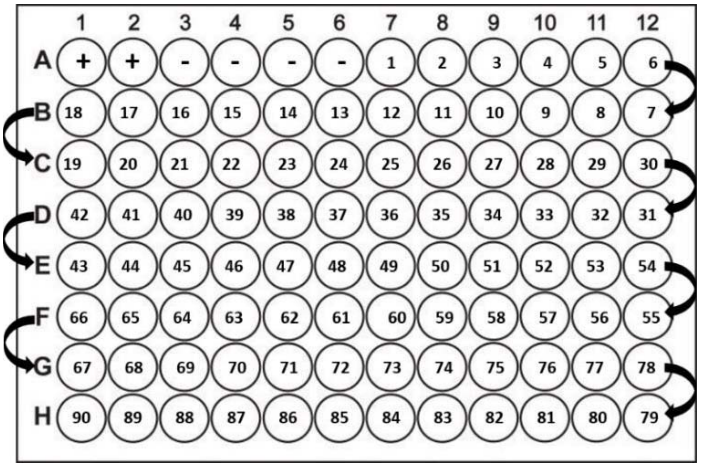


Figure 14: Fraction collection on a 96-well plate.
The separated fractions were collected every 50 s in a meandering pattern. As a positive control (+), pure medium was used corresponding to 100% growth inhibition. For negative controls (-), fully grown cultures were used. Arrows indicate the meandering of fraction collection.

The analysis of the 44 separated extracts led to the identification of several bioactive fracions on the 96-well plates. Positively screened crude extracts and their corresponding retention times are listed in **Table 8**.

In total, 15 crude extracts retained bioactivity after HPLC separation. Due to the use of a reverse phase C8 column, hydrophilic substances eluted at the very beginning and caused inhibitory effects in wells A9 and A10, respectively, thus resulting in two consistently bioactive fractions.[2]

Apart from the strain FF6-H_2O, almost all crude extracts active against Gram-negatives had lost their bioactivity after HPLC separation. The extract of the *Serratia* isolate FF6-H_2O was further separated by HPLC and the obtained fractions were tested against *E. coli*. A broad tailing of anti-Gram-negative bioactivity was observed, even when applying low concentrations of crude extract. Therefore, work on this strain was intensified while the identification of active fractions continued for the remaining 15 crude extracts (see **Table 8**)

These 15 extracts exhibited very different patterns of bioactive fractions. For example, the *C. albicans*-inhibiting MeOH extract of isolate Wild Mid 14 revealed three active fractions (A9/A10 and E7). The activity of fractions A9 and A10 can be regarded as unspecific as mentioned above. The fraction of well E7, which corresponds to fraction 49 (**Figure 14**), can be recalculated with a retention time of around

[2] not listed in **Table 8**

40 minutes and 50 seconds. Often, more than one fraction of an extract showed antimicrobial effects as can be seen in the case of the *M. smegmatis*-inhibiting extract 3MA5 or the *E.coli*-inhibiting extract FF6-H_2O. The latter is one of those bacterial extracts with broad antimicrobial activity identified in the primary screening (**Table 7**). After HPLC separation and microbiological screening of the collected fractions, a broad tailing was observed, as already mentioned above. Active fractions tailed from 18 minutes 20 seconds to 25 minutes (wells C4-C12); moreover, a single fraction in well E2 (36 min 40s) was identified.

The identification of active fractions is a crucial step for further analysis and de-replication of known bioactive compounds by LC-MS and MS-MS. Therefore, those 15 remaining crude extracts were analysed by LC-MS. To combine the calculated retention times for the de-replication process, the LC-MS analysis was performed with the same HPLC protocol and the same type of C8 column. Also the LC instrument was of the same type as the one used for primary separation. Consequently, the retention times of both HPLC separation and LC-MS should approximately be in the same time range.

Table 8: Bioactive fractions of the MeOH crude extracts after HPLC separation.

Isolate	Inhibition	Active well	Retention time					
FF6-H_2O	*E. coli*	C4-C12/E2	18 min 20s	-25 min	36min 40s			
2FM1 d9	*C. albicans*	C1/E10/E11/E12	15 min 50s	43 min 20s	44 min 10s	45 min 00s		
Wild Mid 14 d9	*C. albicans*	E7	40 min 50s					
60 d9	*M. smegmatis*	D4	32 min 30s					
66 d9	*M. smegmatis*	F1/G1/G3/G4	55 min 00s	55 min 50s	57 min 30s	58 min 20s		
80 d9	*M. smegmatis*	D4/H12/H11/H8	32 min 30s	65 min 50s	66 min 40s	69 min 10s		
86 d9	*M. smegmatis*	G12/H8	65 min 00s	69 min 10s				
71 d9	*M. smegmatis*	F12/F11/F10/F8/ G6/G10/H6/H9	45 min 50s	-49 min 10s	60 min 00s	63 min 20s	68 min 20s	70 min 50s
3FM1 d1	*M. smegmatis*	G11	64 min 10s					
3MA3	*M. smegmatis*	B9/B8/B7	8 min 20s	9 min 10s	10 min 00s			
3MA5	*M. smegmatis*	B3-B9/ C3-C8	8 min 20s	-13 min 20s	17 min 30s	-21 min 40s		
3MH1	*M. smegmatis*	G6-G10	60 min 00s	60 min 50s	61 min 40s	62 min 30s	36 min 20s	
2MH3-2 d9	*S. aureus*	E10/F11/F9/F8/F7 F4/F1	43 min 20s	48 min 20s	49 min 10s	50 min 0s	52 min 30s	55 min 00s
2MH1 d1	*S. aureus*	E12/F9/F8/F5/F2	45 min 0s	48 min 20s	49 min 10s	51 min 40s	54 min 10s	
2MH1_PGS d6	*S. aureus*	F9/F8/F5	48 min 20s	49 min 10s	51 min 40s			

Aim 3: Analytic and isolation of interesting candidates

6.4 Liquid chromatography-mass spectroscopy (LC-MS) analysis

During the past 70 years, the structure of myriads of bioactive natural products has been elucidated. Consequently, the rediscovery of known antibiotics is common. This fact underlines the value of de-replication. Liquid chromatography – mass spectroscopy is therefore the method of choice to analyse the composition of crude extracts. The aim was to identify the active fractions obtained by HPLC separation of the crude extracts (see above. By taking into account the possibility of a slight retention time shifts, the LC-MS data were first scrutinised with Bruker the programme “DataAnalysis”. Subsequently, the natural products database "AntiBase” 2014 (Wiley) was searched possible hits corresponding to the major peaks in the metabolite pattern of each extract.

Therefore, retention times of the antimicrobially active fractions were determined according to HPLC separation and screening results. Due to these calculations, it was possible to focus on defined retention time frames in each data set. The AntiBase search did not yeald defined results, i.e. a match with any of the entries compiled in the database. Moreover, because of the complex, labourius test procedure for anti-*M. smegmatis* activity, the focus was set on crude extracts inhibiting *E. coli*, *S. aureus* or, *C. albicans*, respectively.

The extract 2MH3-2 was chosen here as an example for MS analysis. This extract inhibited the growth of *S. aureus*. The major peaks in the base peak chromatogram of this crude MeOH extract (**Figure 15**) were checked for known antimicrobial compounds at the calculated retention times (43 min 20 s as well as the time period from 48 min 20 s to 55 min, see **Table** 8).

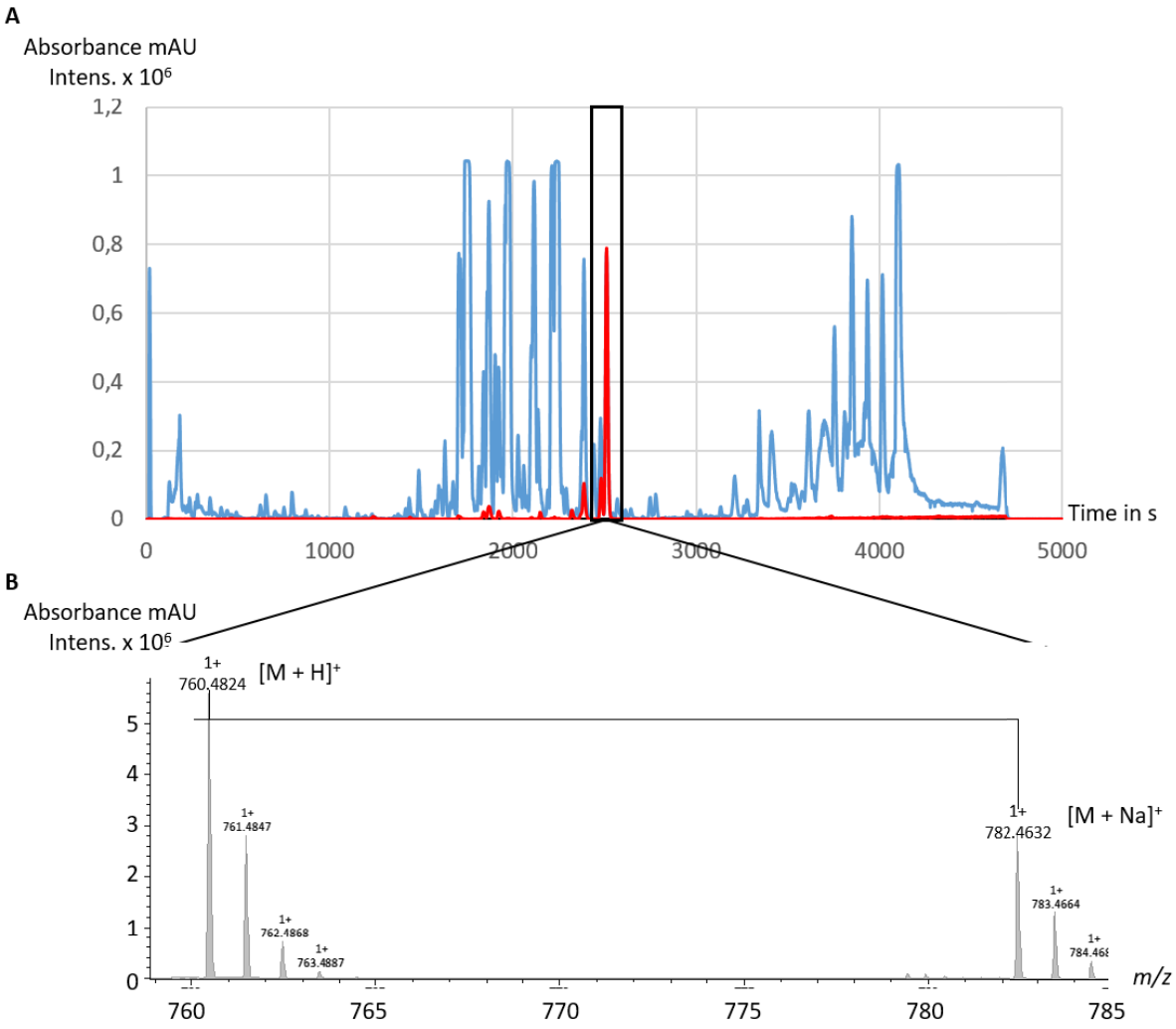


Figure 15: LC-MS analysis of crude extract 2MH3-2.
A: Base peak chromatogram (blue) of the crude extract 2MH3-2 and the extracted ion chromatogram (EIC, red) of *m/z* 760 (±0.5). The major EIC peak with a retention time of 2490 s corresponds to *m/z* 760 ($[M+H]^+$).
B: The magnification of the LC-MS chromatogram in the time range of approximately 2490s exhibited the protonated pseudomolecular ion *m/z* 760.4824 as well as the corresponding sodium adduct *m/z* 782.4682.

By taking into account a slight retention time shift, one major peak could be detected at 41 min 30 s. In contrast, no others were found (see **Table 8**). This peak corresponded to *m/z* 760.4824 ($[M+H]^+$, **Figure 15**). A subsequent AntiBase search revealed a potential candidate corresponding to *m/z* 760.4842 (Δ *m/z* 2.4). According to these search results, an erythromycin analogue with the sum formula $C_{39}H_{70}NO_{13}$ was considerered. However, MS/MS experiments disproved this hypothesis. **Figure S1** displays the fragmentation pattern of the pseudomolecular ion of *m/z* 760.4677. The most notable feature was the multiple loss of H_2O indicating the presence of -OH groups (Δ *m/z* 18). Addtional losses of ammonia (NH_3), corresponding to a mass difference of *m/z* 17, were observed, indicating the presence of $-NH_2$ groups. Differences of *m/z* 28 were shown to correspond to the loss of -CO. Taken togehter, the MS/MS analysis did not support the hypothesis of an erythromycin analogue. Moreover, a core skeleton could not be detected, even after adjusting the collision energy settings.

Due to the missing core skeleton, semi-preparative isolation and subsequent 1- and 2D-NMR were required to complete the structural elucidation of this bioactive metabolite.

Aside from the extract of isolate 2MH3-2, the extract of strain FF6-H_20 was analysed. HPLC separation and LC-MS/MS experiments revealed a pseudomolecular ion *m/z* 397.8521. Moreover, an AntiBase search for this candidate mass did not yield any hits. In contrast, a literature search for bioactive compounds isolated from *S. plymuthica* strains resulted in the hypothesis of zeamine I (*m/z* 397.8527) as the bioactive compound (Masschelein et al., 2013). A set of preliminary MS/MS data seemed to support this hypothesis for zeamine I ($C_{47}H_{93}N_3O_6$). Nevertheless, a subsequent detailed LC/MS and MS/MS investigation could not confirm the initially hypothesised mass of *m/z* 397.8527 and therefore zeamine I was excluded from further considerations. Therefore, semi-preparative isolation and NMR-based structural elucidation were considered.

After refermation, the MeOH crude extracts of strain FF6-H_2O were tested not only against *E. coli* as before but also against *S. aureus* and *P. aeruginosa*. Notably, fractions of different bioactivity against the three pathogens tested have been observed. Antimicrobial effects against *E. coli* were recorded for factions corresponding to a retention time of 18 min 20s to 25 min as well as a single fraction at 36 min 40s. Inhibition of *S. aureus* was observed from 20 min to 20 min 50s and again from 24 min 10s to 25 min (see **Figure 16**). These inhibitory effects on *E.coli* and *S. aureus* were mainly present in the same fractions or in fractions close to each other. In contrast, the inhibition of *P. aeruginosa* was observed in two seperate fractions eluting at a retention time of 35 min and 49 min 10s, respectively (see **Figure 16**). Here, the retention time of 49 min 10s differs extremely from the other retention times of the separated FF6-H_2O crude MeOH. This contradicts the hypotheses of either only a single zeamine derivative or an unknown compound as the active principle. Zeamine is targeting the cell wall unspecifically, which then should result in the same bioactive fractions inhibiting the pathogens tested.

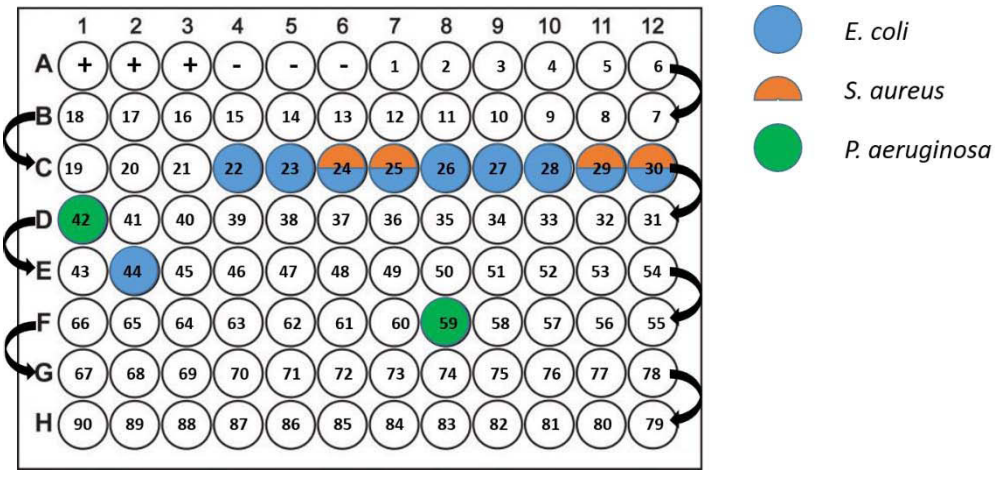


Figure 16: antimicrobial activity of FF6-H_20 after HPLC separation of the MeOH extract.
This scheme illustrates the distribution of fractions with antimicrobial activities against *E. coli*, *S. aureus* or *P. aeruginosa*, respectively Fraction collection was performed as described and indicated in **Figure 14.** The coloured wells correspond to a 100% inhibition of the tested pathogens.

6.5 Flash chromatography

6.5.1 Isolate 2MH3-2

LC/MS as well as MS-MS analysis were not sufficient to identify distinctive pseudomolecular ions in the crude MeOH extract of the *Serratia* isolate 2MH3-2. Therefore, an NMR approach was the only way to identify the bioactive compound. In order to obtain sufficient amounts of purified material for 2D-NMR, the fermentation process had to be scaled up. Due to the fast metabolite and high biomass production of the *Serratia* strain 2MH3-2, a 2 L fermentation was carried out in a 5 L Erlenmeyer flask. After freeze drying, the residue was extracted, yielding 15 g of dried MeOH extract. To further reduce the amount of this crude extract, and to prevent overloading of the flash chromatography column, proteins were precipitated with acetonitrile (MeCN) after redissolving in H_2O. This way, the amount of crude extract was reduced by 40%. Both the aqueous phase and the MeCN precipitate were checked for inhibitory effects on *S. aureus*. Here, the strongest bioactivity was found in the aqueous phase.

The dried aqueous phase of strain 2MH3-2 was redissolved in MeOH and loaded onto Cellite in a ratio of 1:1 (w/w). Afterward, a pre-column was filled with the loaded sample and separated by a gradient as described in Material and Methods.

Due to the reverse phase of the column, hydrophilic compounds elute earlier than lipophilic compounds. Consequnetly, huge amounts of sugars and salts elute in the first 10 min of the purification process (**Figure 17**). As the intensity of the peaks containing these hydrophilic compounds is extremely high, smaller peaks eluting after 10 min are suppressed in their intensity. Due to the previous fractionation of the crude MeOH extract, the polarity of the bioactive fraction could roughly be estimated. Elution of the bioactive compound was previously achieved at 84% MeCN/ 16% H_2O. From this, it was estimated that the bioactive fraction will elute from the flash column at 60% MeCN due to higher flow rate and column size. The fractions from the Puriflash column were collected in a peak-dependent manner; however, the first 10 minutes were collected as single, large fraction containing all hydrophilic substances. **Figure 17A** shows the resulting chromatogram with the dominant peak of hydrophilic substances at the beginning. For a better resolution, the time frame containing substances of medium polarity (15 min to 42 min 30s) is magnified in **Figure 17 B**.

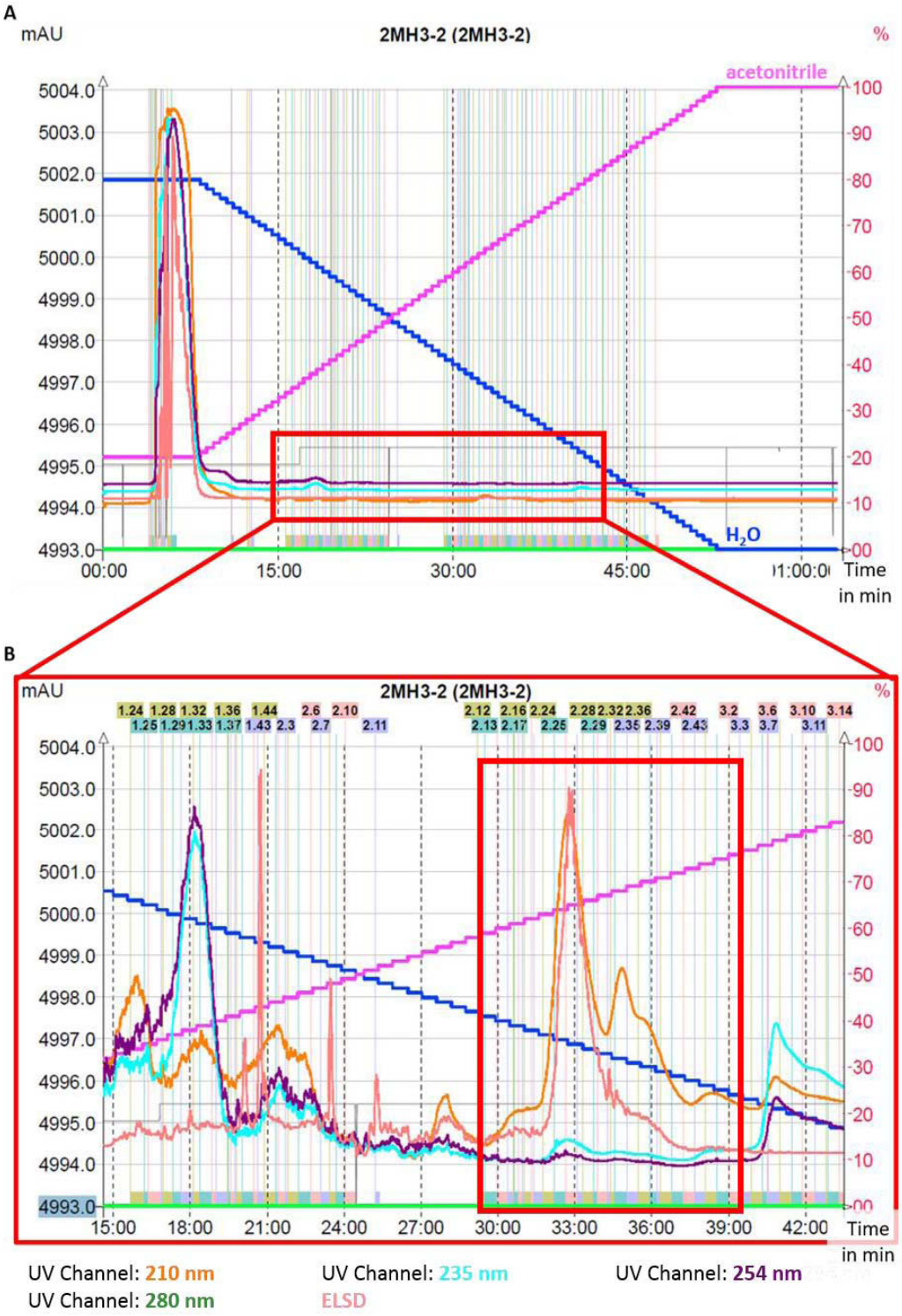


Figure 17: Flash chromatography elution profile of sample 2MH3-2.
A: The elution profile shows a dominant peak from 5 to 10 min. This peak is caused by high amounts of hydrophilic substances e.g. sugars and salts. The high intensity of this peak led to an auto scaling whereby the following peaks of lower intensity are suppressed. This has also be taken into accoun for the estimated elution of the bioactive substance within the range of 60% MeCN.
B: The different scale of the elution profile in the time range from 15 min to 42 min 30s reveals the peaks of lower intensity. The peak (λ=210 nm) with a retention time from 29 min to 39 min 30s (red box =fraction 3) contained the bioactive fraction. The coloured numbers above the profile indicate the respective code for collection tray and tube.

The flash purification yielded four larger fractions which were subsequently retested for *S. aureus* inhibition. As described above, the hydrophilic substances eluting during the first 10 min were manually combined and collected to prevent a possible loss of active substances. After that, the peaks were collected automatically and combined to logical fractions based on the obtained elution profile. Finally, that resulted in **fraction 1** (1.21-1.40), **fraction 2** (1.41-2.10), **fraction 3** (2.11-3.04) and **fraction 4** (3.05-3.22). Beside these fractions, also the waste was collected. After evaporation and redissolving of the eluted material, subsequent bioactivity testing within a serial dilution against *S. aureus* DSM 799 led to the identifiaction of the candidate fraction 3. Therefore, fraction 3 was considered for further purification by semipreparative HPLC. After testing the crude fraction 3 for analytical purity (**Figure 18A**), four additional fractions were generated by HPLC separation (**Figure 18 B**). Subsequently, these four fractions, the original crude fraction and the obtained HPLC waste were tested for bioactivity. Afterwards, fraction 3 was identified as the most active one, with inhibitory effects down to a concentration of 20 µg/mL extract.

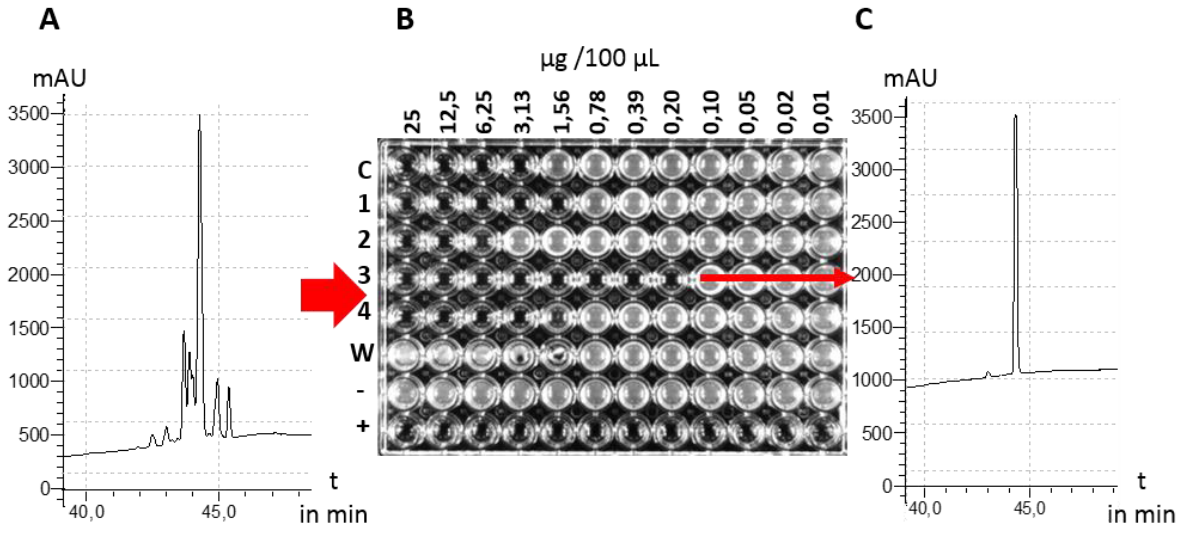


Figure 18: HPLC purification of the bioactive Flash chromatography fraction.
Purification of the Flash chromatography fraction 3 to obtain high purity for NMR analysis. This resulted in four subfractions, which were tested for antimicrobial effects against *S. aureus* DSM799.
A: HPLC elution profile of the active Flash chromatography fraction 3 before HPLC purification.
B: The four subfractions as well as the crude extract (c) and the waste (w) were tested in a serial dilution against *S. aureus* from 25 µg to 0.01 µg/100 µL. This resulted in the identification of subfraction 3 as the most active one against *S. aureus*.
C: HPLC elution profile of the purified subfraction 3. HPLC was performed to check for analytical purity, which is essential for further NMR analysis.

6.5.2 Isolate FF6-H_20

The strain FF6-H_20 was fermented multiple times, extracted and fractionated by HPLC. Fraction collection was performed as shown in **Figure 16** and guided by bioactivity testing. Multiple FLASH chromatography experiments were carried. Fractions were tested for bioactivity without a postive result. Obviously, the activity was lost even though the extracts were positively tested before.

After multiple fermentations the isolate FF6- H_20 seemed to have lost its bioactivity. The crude extract was not able to inhibit any of those pathogens that have been positively tested before. Therefore, the fermentation broth was supplemented with synthetic *N*-(3 oxohexanoyl)-C6-homoserine lactone (2.5 mg/L) to induce the production of secondary metabolites. In contrast to the literature (Masschelein et al., 2013), no induction of antimicrobial activity was observed. This *N*-acyl homoserine lactone (AHL) is known to act as a quorum sensing molecule, thus activating the the biosynthetic gene cluster (BGC) for zeamine production.Furthermore, co-cultivation experiments with *E. coli* were also performed but without success (data not shown).

6.6 NMR analysis

As already outlined above, the de-replication process of the *S. aureus*-inhibiting compound of the crude MeOH extract of Serratia strain 2MH3-2 could not be completed by well-established LC/MS and MS/MS approaches. Therefore, structural elucidation by nuclear magnetic resonance spectroscopy (NMR) had to be attempted. The purified antimicrobial compound displayed a pseudomolecular ion *m/z* 731.4464 ([M+H]$^+$). NMR experiments indicated the presence of a cyclic lipodepsipentapeptide. A peptide of this size has already been described for *S. marcescens* and is known as serrawettin W2 (Matsuyama et al., 1992), i.e. *cyclo*(3-hydroxydecanoyl-D-leucyl-L-seryl-L-threonyl-D-phenylalanyl-L-isoleucyl).

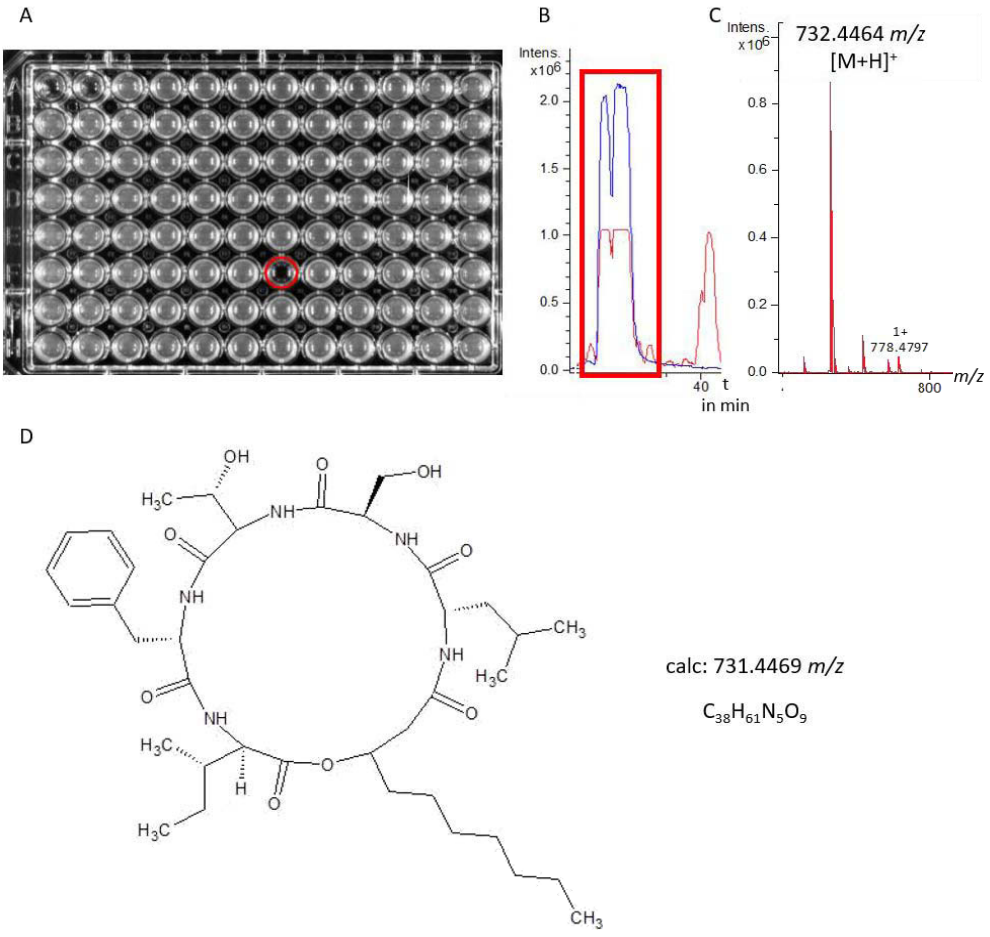


Figure 19: Identification of serrawettin W2.
A: Bioactivity guided identification of the *S. aureus*-inhibiting fraction. The single blank well, circled in red, indicates the antimicrobially active fraction.
B&C: LC/MS and MS-/MS experiments revealed a pseudomolecular ion *m/z* 731.4464 ([M+H]$^+$).
D: The cyclic lipodepsipentapeptide serrawettin W2 was identified by NMR. It consists of a ring of 5 amino acid residues and single fatty acid ester, i.e., *cyclo*(3-hydroxydecanoyl-D-leucyl-L-seryl-L-threonyl-D-phenylalanyl-L-isoleucyl).

The pure compound serrawettin W2 was afterwards tested for its minimal inhibitory concentration (MIC) against both Gram-positive and Gram-negative bacteria (**Table 9**). The clinically highly relevant, methicillin-resistant *S. aureus* (MRSA) displayed the identical MIC value of 4 µg/mL as the other

Gram-positive bacteria tested. Apart from this, the Gram-negative test strains *E. coli* wildtype and the ΔTolC mutant exhibited no susceptibility to serrawettin W2 - the MIC value was higher than 128 µg/mL. The MIC values determined for serrawettin W2 underlined the antibiotic properties against Gram-positive bacteria and extended its known range of activity to MRSA and *L. monocytogenes*.

Table 9: Minimal inhibitory concentrations of pure serrawettin W2 against representative bacteria.

test strain	accession number	type	MIC (ug/mL)
E. coli	ATCC 25922	wild type	> 128
E. coli	ATCC 25922	ΔTolC mutant	> 128
B. subtilis	DSM 10	wild type	4
S. aureus	ATCC 25923	MSSA	4
S. aureus	ATCC 33592	MRSA	4
L. monocytogenes	DSM 20600	wild type	4

Moreover serrawettin W2 has been previously described as nematode repellent by Pradel et al. (2007). Therefore, an ecological role for the compound was hypothesised; and purified serrawettin W2 was tested for nematostatic or nematicidal effects on *Ceanorhabditis elegans*, respectivly (see **Figure 20**). Subsequently, 100% immotility at a concentration of 128 µg/mL was observed. Thus, the effective dosage (ED_{50}) value of 25.27 µg/mL could be extrapolated, based on an exponential trend line. From these results, it could be concluded that serrawettin W2 is nematostatic at 128 µg/mL. However, after seeding the serrawettin-treated nematodes on NGM agar covered with a lawn of *E. coli*, 100% mortality was recorded at a concentration of 256 µg/mL. At a concentration of 128 µg/mL a reduced *C. elegans* population was observed, compared to thos treated with a lower dose.

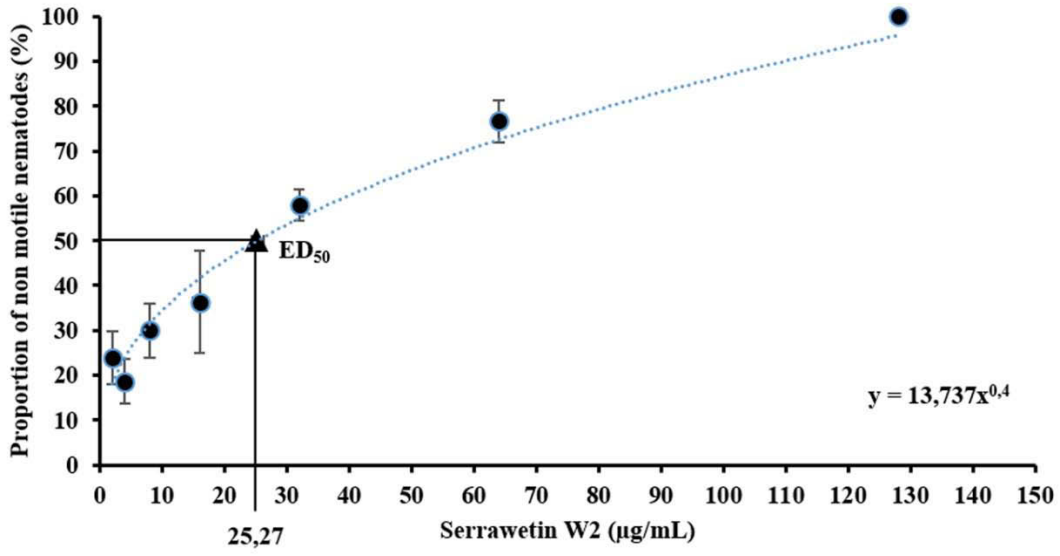


Figure 20: Nematode motility assay with serrrawettin W2
Non-motile nematodes were counted under the microscope, and the percentage of non-motile animals was calculated. The resulting effective dose (ED_{50}) was extrapolated directly from the graph displayed above. Average and standard error are given.

6.7 Bacterial genome sequencing

Genomic DNA of selected bacterial isolates was subjected to whole-genome sequencing using the PacBio technology in order to receive full genomes of so far unidentified, bioactive isolates from the gut microbiome of *N. vespilloides*. Therefore the isolates 2ABFH4, inhibiting *S. aureus* and *P. aeruginosa*, Wild Mid 14, affecting *C. albicans*, and isolate 39, with bioactivity against *E. coli* and *M*. *smegmatis*, were prioritised. Approximately 1.3 to 1.7 gigabases (Gb) of sequencing information was generated per strain and used for *de novo* genome assembly followed by a genome circularization step (**Table 10**). Isolate 2ABFH4 has a genome size of 4.49 megabases (Mb) and gene prediction identified 4,070 putative genes. For the isolate Mid Wild 14, a 3.82 Mb genome was assembled and 3,342 genes were predicted. In contrast, the genome of the isolate 39 was assembled into three contiguous sequences (contigs). The largest contig represents the circular bacterial chromosome with 3.78 Mb and comprises 3,479 predicted genes. The genome additionally includes two extrachromosomal sequences with 63.95 kilobases (kb) and 107.43 kb. Sequence analyses have shown that both sequences most likely represent plasmid sequences. The 63.95 kb extrachromosomal sequence is circular and encodes for 78 open reading frames. For the larger extrachromosomal sequence (107.43 kb) 108 open reading frames were predicted. For this sequence, it was not possible to establish a circularized version using bioinformatics tools. In order to test if its sequence also represents a circular extrachromosomal sequence, PCR and subsequent Sanger sequencing spanning the gap is necessary.

For phylogenetic classification of the three isolates, Sanger sequencing of the 16*S* rDNA region proposed 2ABFH4 to be most closely related to *Providencia rettgeri* (100% identity, ID), Wild Mid 14 to *Proteus mirabilis* (~98-100% ID) and 39 to *Arthrobacter* sp. (~98-100% ID). However, a comparison of the isolates on the whole genome level to sequenced genomes deposited in public data bases revealed that only the isolate Mid Wild 14 and *P. mirabilis* share significant parts of their genomes (conserved DNA = 94.05%) with an overall ID of 99.43%. For the isolated strains 2ABFH4 and 39 the homologous regions with their next sequenced relatives are only 3.92% and 12.84%, respectively.

Table 10: Analysis of the PacBio sequencing effort on 3 selected isolates

Isolate	**2ABFH4**	**Wild Mid 14**	**39**
Sequenced bases [Gb]	1.30	1.55	1.69
Contigs	1	1	3
Genome size [Mb]	4.49	3.82	3.96
G+C content	41.13	38.64	57.53
CDS predicted (protein coding sequences)	4,070	3,342	3,665
Closest genetic relative available	*Providencia rettgeri* FDAARGOS_330	*Proteus mirabilis* AR379	*Glutamicibacter arilaitensis* KLBMP 5180
Conserved DNA	3.92%	94.05%	12.84%
ANI (average nucleotide ID)	86.50%	99.43%	88.47%

6.8 Analysis of biosynthetic gene clusters

Most of the antibiotically active secondary metabolites are produced by polyketide synthases and non-ribosomal peptide synthetases (PKS/NRPS) encoded in biosynthetic gene clusters. The de-replication of the observed bioactivity of the isolates 2ABFH4, Wild Mid 14 and 39 yielded no valuable results. Consequently, a bioinformatics approach was chosen. Whole genome sequencing (WGS) was used toidentify any present biosynthetic gene clusters (BGC). All three sequenced genomes were analysed using antiSMASH, a tool for the automated identification, annotation and analysis of secondary metabolite gene clusters. However, only strain 39 identified as *Glutamicibacter* sp. carries a hybrid type I PKS/NRPS cluster of 55,885 nucleotides. The predicted core BGC contains one type I PKS gene, three NRPS genes, one gene coding for a cytochrome P450-containing protein, one ACPS-type acetyl carrier protein (ACPS) and two thioesterase domains (TE) encoded in individual genes (**Figure 21**). The PKS gene consists of one ketosynthase (KS) domain, one acyltransferase (AT) domain and one peptidyl carrier protein (PCP) domain. The KS domain contains the conserved CSSSL and HGTGT motifs, which are essential for its functionality. One of the NRPS genes only contains one adenylation domain (A) and one peptidyl carrier protein PCP domain; each of the other two contain one A domain, one condensation (C) domain and one PCP domain, respectively. The PKS part of the BGC shares similarities to a BGC from *Arthrobacter* sp. IHBB11108 with an identity of 64%. However, the rest of the hybrid BGC does not share those similarities.

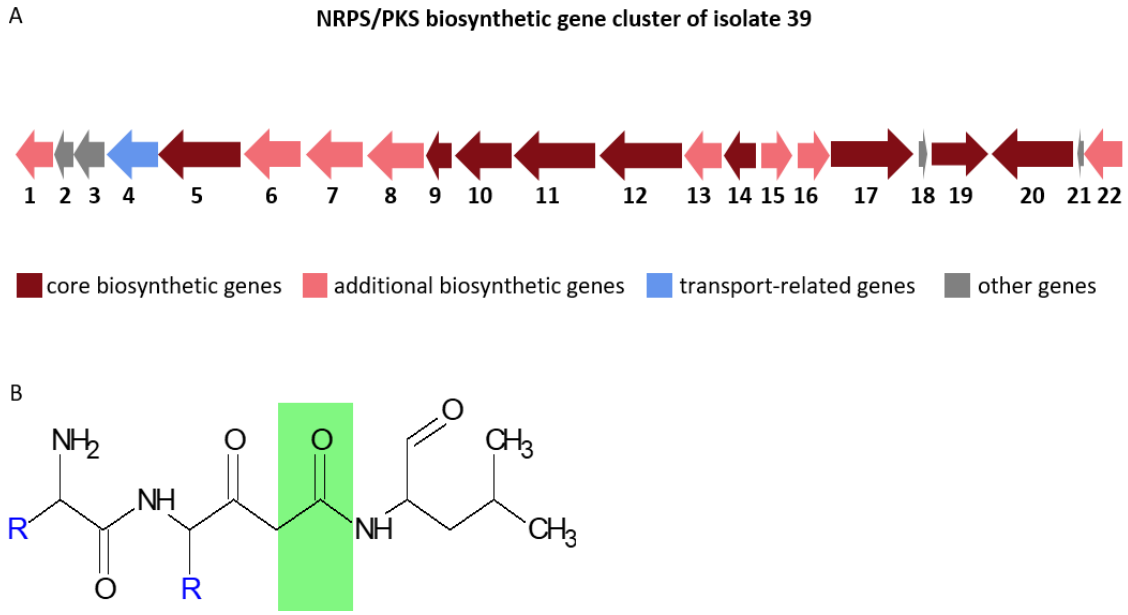


Figure 21: Biosynthetic gene cluster of strain 39.
A: The prediction tool antiSMASH detected a hybrid NRPS/PKS BGC. This BGC displays several core biosynthetic genes as well as additional genes encoding for enzymes associated with the synthesis of an unknown peptide. The predicted BGC shares similarity with an already known *Arthrobacter* BGC. Numbers underneath are explained in **Table 11**.
B: The predicted compound of this gene cluster is build up from three amino acids and at least one keto group (green). Only one of these amino acids can be predicted as leucine.

Table 11: List of proteins encoded in the BGC

Element Number	Protein	% Identity to	Function
1	ATP-grasp domain-containing protein	*Arthrobacter* sp. 69%	
2	glyoxalase/bleomycin resistance/dioxygenase family protein	*Arthrobacter* sp.86%	
3	hypothetical protein	*Arthrobacter* sp. 65%	
4	MFS transporter	*Arthrobacter* sp. 75%	
5	S9-family peptidase	*Arthrobacter* sp. 64%	
6	hypothetical protein	*Arthrobacter* sp. 65%	
7	ATP-grasp domain-containing protein	*Arthrobacter* sp. 60%	
8	ATP-grasp domain-containing protein	*Arthrobacter* sp. 75%	
9	Thioesterase	*Arthrobacter* sp. 69%	TE
10	amino acid adenylation domain-containing protein	*Arthrobacter* sp. 63%	A and PCP
11	type I polyketide synthase	*Arthrobacter* sp. 64%	KS, AT and PCP
12	non-ribosomal peptide synthetase	*Arthrobacter* sp. 59%	C, A and PCP
13	4'-phosphopantetheinyl transferase superfamily protein	*Kitasatospora purpeofusca* 46%	ACPS
14	cytochrome P450	*Arthrobacter* sp. 68%	
15	hypothetical protein	*Arthrobacter* sp. 69%	

Element Number	Protein	% Identity to	Function
16	acyl-CoA dehydrogenase	*Arthrobacter* sp. 52%	
17	non-ribosomal peptide synthetase	*Arthrobacter* sp. 58%	C, A and PCP
18	*N*-acetylmuramoyl-L-alanine amidase	Oscillatoriales (cyanobacterium) 47%	
19	Thioesterase	*Arthrobacter* sp. 66%	TE
20	S9-family peptidase	*Arthrobacter* sp. 63%	
21	Unknown	unknown	
22	GNAT family N-acetyltransferase	*Arthrobacter* sp. 79%	

While the automated structure prediction by antiSMASH always has to be reviewed critically, the basic chemical features of the compound encoded by the BGC can be deduced from the amino acid sequence. The presence of three A domains already indicates the incorporation of three amino acids into the final molecule. Nevertheless, the specificity prediction of the A domains resulted in only one defined result, namely leucine. Ultimately, the amino acid prediction and the resulting structure remain vague. Consequently, NMR analysis is required to advance this structural elucidation.

Discussion

Aim 1: Isolation, cultivation and identification of the gut microbes

7.1 The beetle

For the start of the project it was necessary to obtain burying beetles. Therefore, catching of living, wild animals was prioritiesed. As the project started in April 2015, also laboratory-hatched *N. vespilloides* had to be used due to cold weather, which prevented a successful collection in the natural habitat. In the period from May to August, collection of the beetles with a carrion-baited trap was successful. Nevertheless, *N. vespilloides* appears to have a certain temperature preference for mating and breeding. In high temperatures of August, catching was often not successful. The literature describes a similar effect of the appearance of burying beetles. It also indicates a time- and species-dependent breeding behaviour for burying beetle communities (Scott, 1998). This may explain the decreasing success of catching in the late summer months. Moreover, the area of distribution is specific to different *Nicrophorus* species. *N. vespilloides* has been described as a species commonly found in beech forests. The distribution of baited traps in this study was covering several types of areas, from pinewood to grassland. Almost all beetles were caught in the widely distributed beech and mixed beech/oak forests in the vicinity of Giessen/Germany. The same areal distribution was already described by Pukowski in 1933.

Rearing of the beetles was possible under laboratory conditions but breeding was not successful. The larvae hatched and migrated but the development stopped in the phase of pupation. This might be an effect of aridity, which than inhibited the further developement of the pupae. The use of local soil material low in organic matter could be a reason for this (Rawls et al., 2003). Organic materials have a higher ability to bind humidity and to keep the soil moist. To prevent a change in both, the microbial community of the soil and the microbiome of the beetles, the soil material was not artificially changed. The method of *N. vespilloides* rearing was previously described by Jacobs et al. (2014). Beetles were kept at a constant temperature of 20°C and a day/night shift of ~ 15:9 hours. Most of the beetles in this study were kept in a dark incubator, which might influence the beetles' development. Attempts of rearing the beetles at room temperature on the bench top resulted in a quick dehydration of the soil. A humidity of 80% as described for a peaty substrate (von Hoermann et al., 2013) was not achieved. This leads to the conclusion that the use of local soil, low in organic matter, is not suitable due to its low water capacity and the resulting humidity issues.

Finally, the rearing of the beetles was stopped because of the multiple issues described above. Humidity and daylight control would require the use of a modern climate chamber, which was not available for this project. Moreover, the project was focussed on analysing the native mirobiome of the beetles. Therefore a constant supply of high nubers of beetles was not.necessary. It appeared that

catching during the warm period of the year and subsequent direct dissection was sufficient to provide the required material. During the winter time, laboratory-hatched beetles from the Max-Planck Institute for Chemical Ecology (Jena) were used, whenever necessary.

7.2 The microbiome

The key to a successful investigation of the antimicrobial potential of the *N. vespilloides* gut microbiome was the isolation and cultivation of the microbes hosted. In general, the cultivation of mircoorganisms is limited by environmental parameters i.e. temperature, pH-value, oxygen supply and nutrient composition including the availabilty of micro nutrients and trace elements. However it appears challenging to mimic these parameters under laboratory conditions. This fact is the reason for the so-called "great plate count anomaly" (Staley and Konopka, 1985). This phenomenon was described by the finding of a large discrepancy of bacteria observed under the microscope in contrast to those growing in culture under laboratory conditions (Razumov, 1932). Today, molecular methods, e.g. 16*S* rRNA gene amplification and sequencing provide insights into the vast diversity of microbial communities without the restrictions caused by cultivation (Head et al., 1998). Based on this knowledge, it was estimated that less than 1% of the microbial kingdom can be cultivated. Modern cultivation techniques, e.g. the use of the iChip, were recently shown to increase this number by cultivating bacteria in its natural habitat under environmental influences (Nichols et al., 2010).

Cultivation approaches have beem performed previously in other burying beetle species. However, they were focused on the medically important bacteria (Solter et al., 1989; Berdela et al., 1994). The value of these studies was, however, impaired by the use of standart media for clinical microbiology. The first cultivation-independent study on the microbiome of burying beetles (*Silphidae*) unveiled the 50 most abundant bacterial species, based on partial 16*S* amplicon sequences (**Figure 22**) (Kaltenpoth and Steiger, 2014). Also, the *N. vespilloides* hindgut discussed here was considered in this study. The hindgut composition of the *Silphidae* investigated was dominated by the phyla of *Firmicutes* and *Proteobacteria*, whereas *Bacteriodetes* and *Fusobacteria* were less abundant. The most abundant families in the phylum *Firmicutes* were identified as *Enterococcaceae*, *Clostridiaceae* and *Ruminococcaceae*, next to *Xanthomonadaceae* and *Enterobacteriaceae* of the phylum *Proteobacteria*. Kaltenpoth and Steiger underlined the remarkable differences in the microbiota composition of the *Silphidae* with the least diversity found in the hindgut of *N. vespilloides*. Most of the sequenced operational taxonomic units (OTUs) of *N. vespilloides* were *Gammaproteobacteria*. The most abundant families found were the *Xanthomonadaceae* and *Enterobacteriaceae* and the *Enterococcaceae* of the phylum *Firmicutes*.

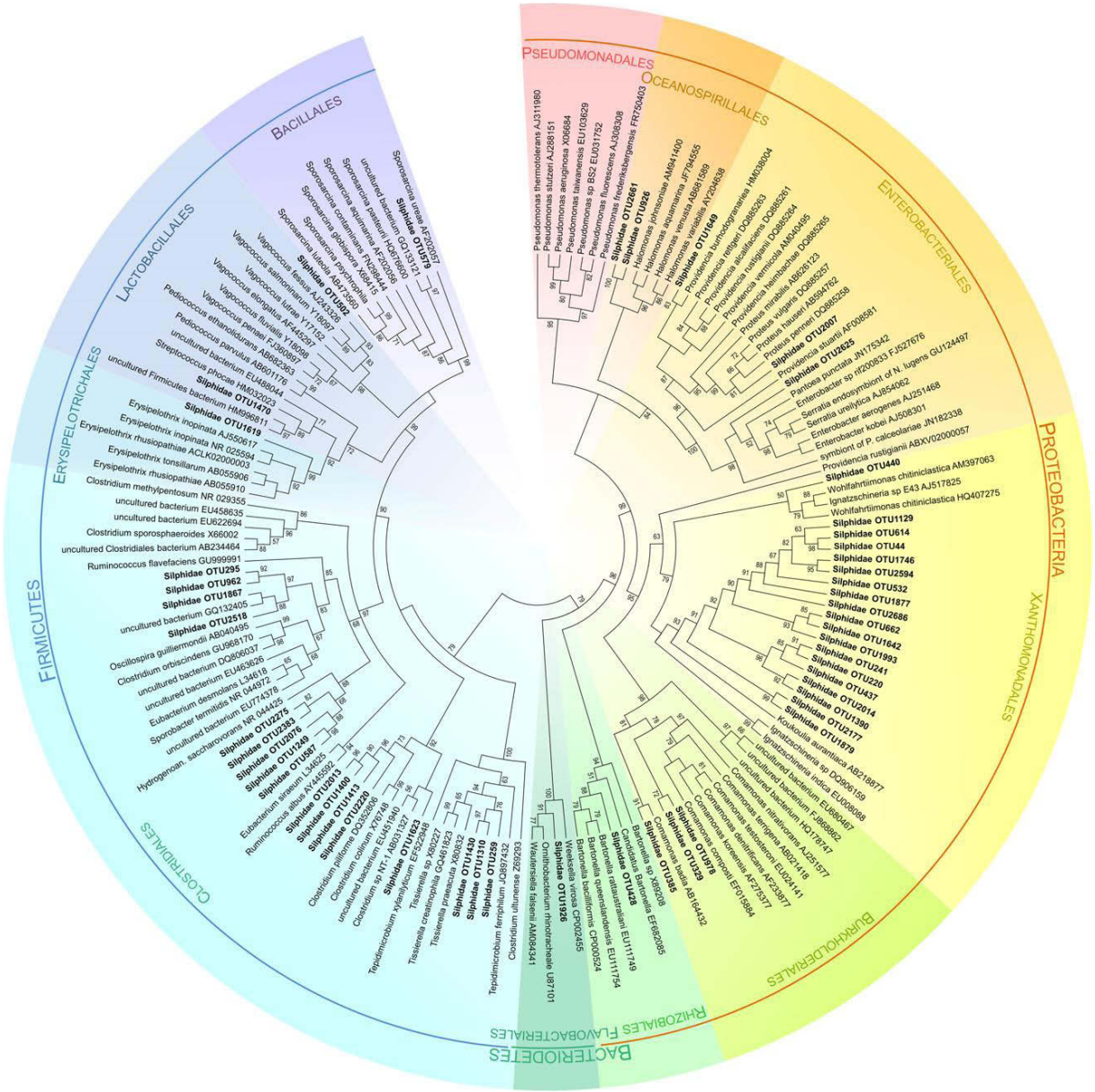


Figure 22: Sequence-based analysis of the *Silphidae* microbiomes.
The figure displays the 50 most abundant operational taxonomic units (OTUs) of the burying beetles (*Silphidae*) as reported by Kaltenpoth and Steiger (2014). The phylogenetic analysis was based on partial 16*S* rRNA gene sequences of a length of 300 bp (amplicon). Phylogenetic sections were coloured to distinguish between bacterial orders and phyla.Figure reproduced with permission of the journal.

Recent investigations on *N. vespilloides* were gained more detailed knowledge on the microbial gut community. Vogel et al. (2017) were able to specify the bacterial composition of both midgut and hindgut. Moreover, they performed fluorescence *in situ* hybridization (FISH) of the major bacterial taxa and were able to localise them in the gut. Their results supported the earlier findings of Kaltenpoth and Steiger (2014) who noticed a high abundance of the *Enterococcaceae* (phylum: *Firmicutes*), especially of the genus *Vagococcus*. Also, the family *Enterobacteriaceae* (phylum: *Proteobacteria*) was present, dominated by the genera *Morganella*, *Providencia* and *Proteus* as well as by the family *Xanthomonadaceae* (order: *Xanthomonadales*). FISH analysis illustrated the high bacterial load in the

gut. Moreover, the use of taxon-specific probes proved the high abundanc of the genera *Vagococcus*, *Morganella*, *Tissieralla*, *Providencia* and *Proteus* as well as of the family *Xanthomonadaceae*.

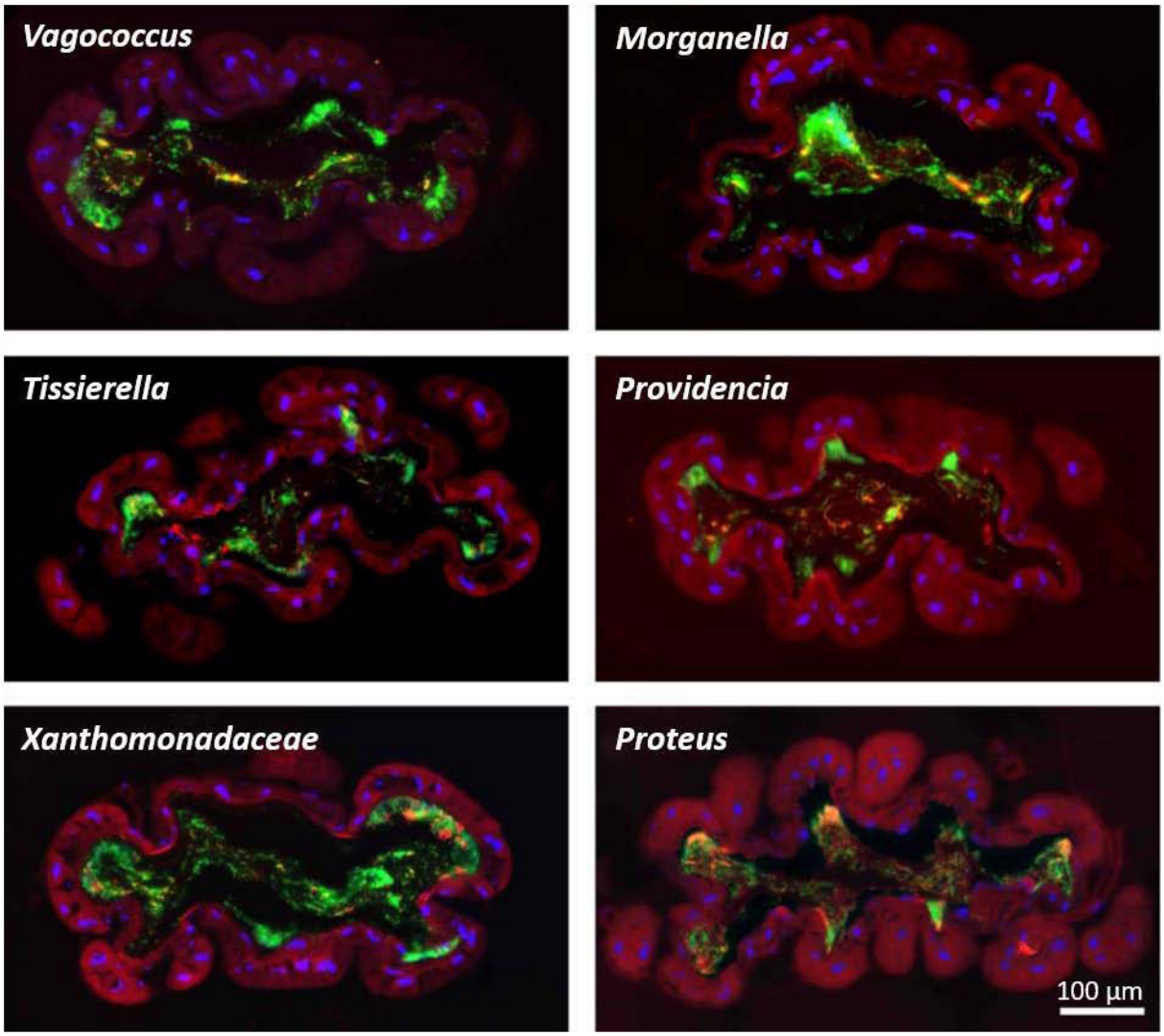


Figure 23: Fluorescence *in situ* hybridization of the *N. vespilloides* hindgut.
A general bacterial probe (green) was used to stain the microbiota inside the gut. Additionally, taxon specific probes (red) were used to colour the six major bacterial taxa. DAPI staining was performed to colour the gut epithelia (Vogel et al., 2017). Reproduced with permission of the journal.

The results of this cultivation-based study are similar to those published by Kaltenpoth and Steiger (2014) and Vogel et al. (2017):

The genera *Proteus*, *Morganella* and *Providencia* of the order *Enterobacteriales* (phylum: *Proteobacteria*) were among the five most abundant cultivates, followed by *Serratia* and *Hafnia*. Additionally, the family *Xanthomonadaceae* were cultivated in high abundance. Most of the bacteria isolated belong to the genus *Stenotrophomonas*. This genus was, next to *Serratia* (order: *Enterobacteriales*), most frequently isolated, followed by the genus *Hafnia*. *Serratia* has not been observed as a major component of the gut microbiota by Vogel et al. (2017). Nevertheless, Kaltenpoth and Steiger (2014) could investigate one of the 50 most abundant *Silphidae* associated

OTUs clustering next to *Enterobacter* and *Serratia*. Moreover, Berdela et al. (1994) was able to isolate *Serratia* from the gut of different species of burying beetles. Recently published data by Shukla et al. (2018) confirm the isolation of *Serratia* from the *N. vespilloies* gut. Surprisingly, in this study *Providencia* was less present than indicated by previous work of Vogel et al, (2017), for comparison see **Figures 9** and **23.** On the other hand, a higher abundance of *Pseudomonas* (order: *Pseudomonadales*) was isolated from the gut. In none of the previous studies *Pseudomonas* has been reported as one of the dominant genera in adult beetles. So far, *Pseudomonas* has only been reported from the larval gut of *N. vespilloides* (Vogel et al., 2017). The presence of the genus *Hafnia* has only been described for other carrion beetles (Berdela et al., 1994) but not for *N. vespilloides*. FISH analysis corroborate the high quantity of *Vagococcus* (order: *Lactobacillales*) in the gut. This is further supported by the results of the cultivation approach in this study. Within the *Bacilli*, *Vagococcus* was the most prominent genus cultivated. In addition, few unspecified bacteria (less than 98% identity via BLASTn) were cultured. Most of these share 16*S* sequence similarity to sequences in the NCBI database, which resulted from 16*S* amplification sub cloning efforts. Hence, no physiology or phylogenetic investigation has been previously performed.

The genus *Proteus* (order: *Enterobacteriales*) is widely distributed in nature and can be found in soil, water and the human gut (Mobley and Belas, 1995). Furthermore, species of this genus can cause urinary tract infections. *Proteus* is notorious for the ability of swarming (Hoeniger, 1964), which has been intensively studied. Evidence for the swarming of *P. mirabilis* by the extracellular signal induction of putrescine has been investigated (Sturgill and Rather, 2004). Putrescine is a biogenic amine, which is produced enzymatically by bacterial decomposition of the amino acids ornithin and arginin, respectively (Wunderlichova et al., 2014). As described in the result section (**chapter 6.3.1**), swarming and overgrowing by *Proteus* isolates caused difficulties in the isolation of other microorganisms. Swarming was reduced by lowering the nutrient content of the growth medium to 50%. The strategy of decreasing the amount of available nutrients inhibited the swarming of *Proteus* efficiently. This finding also underlines the hypothesis that swarming can be initiated by the presence of glutamine (Allison et al., 1993) which is also a constituent of the protein-rich BHI medium.

Providencia (order: *Enterobacteriales*) is another globally distributed genus. It is known for causing nosocomial as well as urinary tract infections. This genus comprises a number of species with antibiotic resistances (Stock and Wiedemann, 1998). Moreover, few *Providencia* species have been reported as insect pathogens in *Drosophila melanogaster* (Galac and Lazzaro, 2011).

The genus *Hafnia* (order: *Enterobacteriales*) has previously been isolated from carnivorous animals, e.g predatory birds and insects (Janda and Abbott, 2006). In rare cases, *Hafnia* was also documented as a human pathogen (Ramos and Damaso, 2000; Gunthard and Pennekamp, 1996;

Albert et al., 1991). Moreover, it was reported as a resource for green chemistry and the production of 1,3-propanediol (Drozdzynska et al., 2014).

Morganella with its single species *Morganella morganii* has been isolated from a vast variety of sources, e.g. animals like dogs, chicken or snakes and human faeces. *M. morganii* was reported as a bacteriocine producer, biosynthesising morganocin (Coetzee, 1967).

Vagococcus of the order of *Lactobacillales* was represented in this study by a single species, *V. fessus,* which has been isolated for the first time from marine mammals (Hoyles et al., 2000). Other vagococci, for example, have been obtained from wasps (Killer et al., 2014) and infected fish (Schmidtke and Carson, 1994).

Serratia isolates were frequently cultivated in this project. Members of this genus have been isolated from a vast variety of environments, comprising air, water and soil. Moreover, strains associated with plants and insects are known (Grimont and Grimont, 1978). In the 1970s, *Serratia* gained some attention due to field trials of the US government on civilian population that were aimed at collecting data on the use of possible bioweapon agents in public places (Mahlen, 2011). Beside this, the species *S. marcescens* is a well-studied insect pathogen (Flyg et al., 1980). This species is mostly recognised by its red colonies. This colouration is caused by the red pyrrole alkaloid prodigiosin (Williams, 1973). However, none of the *Serratia* isolates cultivated here displayed this typical red pigmentation. In fact, *S. marcescens*, *S. proteamaculans*, *S ficaria*, *S. plymuthica*, *S. liquefaciens* and other *Serratia spp.* were isolated multiple times. Subspecies diversity and accuracy of the identification based on 16*S* analysis are limited. Consequently, species diversity remains unknown.

In addition to *Serratia*, the genera *Pseudomonas* (order: *Pseudomonadales*) and *Stenotrophomonas* (order: *Xanthomonadales*) were frequently isolated in high abundance. The former can be found ubiquitously but is also known as a pathogen of animals, humans and plants (Moore al., 1996). *Pseudomonas* species are able to process a large variety of complex organic compounds, e.g the hydrolysis of fats (Goldman and Rayman, 1952). Due to the formation of lipases (Gilbert, 1993) and the ability to metabolise complex carbon sources, gut and diet of the beetles appear to be a suitable habitat for the isolated species *P. brenneri*, *P. extremorientalis*, *P. fluorescens*, *P fragi* and *Pseudomonas* sp. .

The genus *Stenotrophomonas* has been orginally been described as *Pseudomonas*, then hosted in *Xanthomonas* and finally reclassified in *Stenotrophomonas* (Ryan et al., 2009). The main environmental sources for *Stenotrophomonas* are soil and plants, although the genus can be found in almost every environmental niche. Members of the *Xanthomonaedaceae* can grow in a broad temperature range; and several species are reported as oil-and petroleum-degrading (Chang and Zylstra*, 2010). Notably, the genus is able to utilise a broad range of C- and N-sources. It is also known to produce the plant hormone indole-3- acetic acid (Park et al., 2005), thus stimmulating

the growth of both shoots and roots. Apart from that, *S. malthophilia* is an emerging human pathogen, typically causing respiratory tract infections (Brooke, 2012). It has also been reported as a source of a highly active keratinase (Cao et al., 2009). *S. malthophillia* was the most frequently isolated species of this genus. Being associated with plants and soil constitutes, a high potential of colonizing the gut of a soil associated beetle can be hypothesised. The keratinolytic activity can be beneficial for the beetle whilst dealing with the depilation of the carrion. Moreover, the ability to handle lipid-rich substrates is highly valuable for a gut symbiont of *N. vespilloides*.

Alongside the bacteria, yeast and fungi were also cultivated successfully. Notably, yeasts of the genus *Yarrowia* (order: *Saccaromycetales*) are highly abundant in the rectum of *N. vespilloides*. The spectrum of isolatied species included *Y. divulgata* and *Y. lipolytica*; however the majority of isolates were *Y. lipolytica*-like species. *Y. lipolytica* is intensively studied yeast that is capable of degrading hydrophobic substances such as fatty acids, fat and oil (Fickers et al., 2005). It was shown that species of this genus are transferred to the carcass, thereby lowering abundance of soil-borne *Candida* species (Shukla et al., 2018). Besides *Yarrowia*, Shukla and colleagues reported on the presence of *Candida* on the tended carcass. Nevertheless, they also admitted the possibility of contamination due to its abundance in soil. In this context, it appears that *Yarrowia* species are able to outcompete other soil-associated yeasts on the tended carcass. Thus, these authors postulated a major contribution to carcass preservation.

The microbiome of *N. vespilloides* shares similarities to that of sarcophagus flies as already stated by Vogel et al. (2017). A large number of antibiotic resistant bacteria were found in the beetles' gut, including numerous species that are potentially pathogenic to humans. Beside the genus *Tissierella*, all other dominant taxa found by Vogel et al. (2017) were detected in this cultivation approach. *Tissierella* is an obligately anaerobic genus of Gram-negative bacteria (Farrow et al., 1995). In this project, anaerobic cultivation was performed less prioritised. The anaerobic jar cultivation performed, supported the growth of facultatively anaerobic and microaerophilic bacteria. Those can use fermentation and anaerobic respiration but are often able to switch to oxygen dependant respiration. A lot of bacteria classically linked with soil or roots, e.g. members of the orders *Actinomycetales* or *Rhizobiales* (Guerrero et al., 2005), were isolated. Alongside, a large community of *Lactobacilli* and *Enterobacteria* was explored. A lot of the bacterial species e.g. *Serratia marcescens* have been reported as insect-associated – either as gut symbionts or pathogens (Broderick and Lemaitre, 2012). Moreover, lactobacilli are known as producers of bacteriocins (Martínez et al., 2016) which may help the beetle to preserve the carcass of the carrion by their antimicrobial properties.

Together with the bacteriocin-producing *Lactobacilli,* other natural products (NP) producing genera were cultivated. Most genera of the order *Actinomycetales* have been reported as prolific sources of bioactive antimicrobial compounds, especially the filamentous genus *Streptomyces*. The latter has

been screened for NP production since the early 1940s and have been exploited a source of a remarkable number of antibiotics on the market (Watve et al., 2001). The abundance of the order of *Actinomycetales* in this study was relatively low. In this context it has to be considered that most species within this order are soil-borne spore formers. Consequently a count low in *Actinomycetales* corresponds to neglectable cross-contamination by soil- and air-borne propagules of this order.

It is also noteworthy that the microbiome of *N. vespilloides* contains a large group of antimicrobial-resistant bacteria, which have already been reported by Shukla et al., (2018). Resistances towards antibiotics are a naturally occurring phenomenon. In order to survive, any antibiotic-producing organism has to protect itself against self-harming. (Hopwood, 2007). Therefore, the bioinformatics approach on finding new natural products includes the screening of antibiotic resistances next to BGC analysis (Thaker et al., 2013), thus taking into account the relevance of these resistances. Nevertheless, a more classical approach of screening for bioactivities was chosen to uncover the antimicrobial potential of the cultivated microorganisms in this project.

Aim 2: Fermentation, chemical extraction and antimicrobial screening

7.3 Antimicrobial bioactivities

All bacteria and *Yarrowia* isolates were subsequently screened for antibacterial and antifungal activity. Therefore, bacterial isolates were selected based on the 16*S* best BLASTn results. From each species, at least one representative isolate was chosen and considered for fermentation. Because of practicability and common use, it was tried to obtain full 16*S* rRNA gene coverage with the primer set 27F and 1492R. This resulted in a potential maximal read length of 1465 bp and therefore a high coverage of the 16*S* gene (Janda and Abbott, 2007). Generally, this approach is limited by the insufficiency to distinguish between sub-species or, in some cases, even species (Stackebrandt and Goebel, 1994). Hence, it is possible that potentially interesting isolates were not consiedered and thus remained undetected.

In this study, a broad spectrum of bacteria was screened for antimicrobial effects, without preference for those, taxa (e.g. *Actinomycetales, Burkholderiales* and *Bacillales* [Fickers, 2012; Challinor and Bode, 2015]) that have previously been exploited as producers of antimicrobial compounds.

To evaluate the potential of the microbes to produce antimicrobially active secondary metabolites, a kinetic strategy was chosen for the fermentation:

In order to enduce nutrient limitation, bacteria were grown up to 9 days until they reached stationary growth phase (Hopwood, 2007). This strategy obviously promoted the biosynthesis of antimicrobially activity secondary metabolites. As can be concluded from **Table 6**, the majority of extracts displayed antimicrobial activity, which increased by time. Cultures, e.g. strain 89 (*Enterococcus avium)* or strain FF6-H_2O (*Serratia plymuthica*), were inhibiting less of the tested pathogens when harvested after day 1 of fermentation. Afterwards, the spectrum of antimicrobial activity changed. During that time, cultures might have reached nutrient limitation and entered the stationary growth phase. These effects have been summarised and discussed not only for *Streptomyces* but also for *Enterobacteriaceae* (Chater and Mervyn, 1997). The same effect was observed *vice versa* with bacteria producing antimicrobials in the early stage of growth. Once entering a later growth phase, no inhibition of the test pathogens could be observed, anymore. The MeOH extract of isolate 16 (*Serratia proteamaculans*) displayed antimicrobial activity against *M. smegmatis* after day 1 of fermentation. After the following 8 days of cultivation this inhibiting effect was lost (**Table 6**).

Despite several decades of antibiotic research, the question ofhow bacteria can be induced and optimised for antimicrobial compound production has yet not been fully resolved. Nevertheless, versatile strategies for induction of bioactive secondary metabolites can be applied (Knight et al., 2003; Onaka et al., 2011). The missing information on environmental parameters of the beetle's gut decreased the chance to improve the conditions for secondary metabolite production. Hence, the

fermentation in complex media (BHI and TSB) was performed to observe the antimicrobial capacity of the bacterial isolates. Nevertheless, media selection and environmental parameters are known to exert a tremendous impact on secondary metabolite induction (Scherlach and Hertweck, 2009). The complex physiological conditions in the gut such as changing pH-values and oxygen distribution as well as nutrient degradation are important parameters, although dificult to reproduce artificially. The variation of different media can be an option for the optimisation or induction of antimicrobial secondary metabolite production (Bocker, 1978).

After fermentation and chemical extraction, HPLC separation and fraction collection of the crude extracts were performed. Subsequently, after confirmation of bioactivity, LC/MS-and MS/MS-based de-replication was conducted.However it appeared that most of the crude extracts stored in MeOH at 4 C completely lost their antimirobial activity. This unexpectedloss of bioactivity required refermentation (**Table 7**) and chemical extraction. Surprisingly, extracts of $\leq$ 3 months of age remained active, whereas older ones became inactive. Consequently, extracts must not be stored in MeOH for prolonged periods of time in order to retain their antibiotic activity. Afterwards, extracts where evaporated and stored at 4°C to ensure a safe storage.

To limit the rediscovery of antimicrobial metabolites, LC/MS based de-replication with the natural products database “AntiBase” is of high value. Therefore, it is essential to lower the complexity of the crude extracts by HPLC separation and perform LC/MSexperiments. After biological testing of the separated extracts, the bioactive fractions can be scrutinised for known antimicrobials by LC/MS. In theory, the retention time should not differ too much when using the same experimental setup. All extracts that retained their bioactivity were analysed by LC/MS, but no hits inAntiBase were found. Surprisingly, most of the crude extracts revealed a HPLC elution profile devoid of major peaks in the calculated retention time window. Subsequent analysis of serrawettin W2 revealed a considerabele retention time shift of retention times observed during HPLC and LC/MS analysis. Therfore, it is hypothesised that the dereplacation of antimicrobial activies failed due to this shift in retention time. Thus, the discussion of the bioactivities observed is mainly based on literature data.

Another omnipresent phenomenon in natural product research is the sudden loss of antimicrobial activity because the producing organism ceased the biosynthesis of an active compound. In this study, this phenomenon was observed with the majority of extracts active against Gram-negatives.

As already mentioned above, the strategies for induction of the biosynthesis of secondary metabolites are diverse. Nutrients, vitamins and other precursors, homoserine lactones (Demain, 1998) or even the absence of another microbial strain can support or interrupt such induction (Mearns-Spragg et al., 1998; Pettit, 2009). Furthermore, other studies showed that cell-free supernatants can function as an appropriate inducer of antimicrobial production (Burgess et al., 1999).

In summary, most of the observed bioactivities were directed towards *M. smegmatis*. This species is known to be a highly sensitive substitute for screening anti-tuberculotics. However, it has to be drawn into account that it may yield to many positive hits. Therefore, the discrimination between positive hits and false positives, which is only possible by screening *M. tuberculosis*, remains inevitable (Mitscher and Baker, 1998).

Additionally, wild type *E. coli* as well as a ΔTolC mutant of *E. coli,* were selected as test strains. Due to inconsistent inhibition results in the screening process, the ΔTolC mutant had to be excluded from the results afterwards (**Apendix**). TolC is, as well as the multidrug efflux pump element AcrAB, involved in the expulsion of antimicrobial compounds (Koronakis et al., 2004). The inhibition of this efflux pump generates hypersensitive *E. coli* screening strain (Augustus et al., 2004). Penetration of the Gram-negative cell membrane is one of the major challenges in antibiotic drug discovery and developement (Pages et al., 2008). Therefore, potential drugs inhibitng the ΔTolC *E. coli* strain would provide options for a combination with, e.g. AcrAB-TolC efflux pump inhibitors such as pimozide (Bohnert et al., 2013).

Overall, extracts inhibiting Gram-negatives were scarce and derived mainly from *Enterococcus* and *Serratia* species as described previously. The inhibition of *E. coli* often co-occurred with that of *P. aeruginosa*. For *Enterococus faecium*, this has previously been reported (Zheng et al., 2015) and is similar to the antimicrobial activities observed here. Additionally, these antimicrobially active crude extracts from *Enterococcus* species frequently also inhibited *Candida albicans*. This is in contrast to the literature hitherto published in this field. *Candida*-active antimycotics derived from *Enterococcus* are rare but have been mostly identified and reported as one protein with activity on multi-drug resistant strains (Shekh and Roy, 2012). Besides that, numerous bacteriocins of *Enterococcus* species have been reported (De Vuyst et al., 2003).

The red pigement prodigiosin is the most prominent antibiotic compound of the genus *Serratia*. Species producing prodigiosin are easily detectable by their phenotype (Williams, 1973; Lapenda et al., 2015). In this study, none of the *Serratia* isolates formed a red pigment. It has been described, that the induction of prodigiosin biosynthesis is mediated by quorum sensing (Thomson et al., 2000). Environmental parameters such as temperature and nutrients may also affect the induction. Notably, non-pigmented clinical isolates devoid of prodigiosin production are commonly recovered from human specimens (Grimmont & Grimmont, 1978). Apart from prodigiosin, other antimicrobially active secondary metabolites have been reported from this genus such as a β-lactame antibiotics (Parker et al., 1982) or peptide antibiotics e.g. althiomycin (Gerc et al., 2012).

Several unspecified isolates displayed antimicrobial activity. Most of these were active against *M. smegmatis,* but some were also inhibiting *Candida* and *Pseudomonas aeruginosa*, respectively. Because of the numerous antimicrobial effects against *M. smegmatis* and the necessity of a

sophisticated bioassay to confirm the hits, *M. smegmatis* activities were no longer considered for screening.

Unspecified bacteria and strains not related to those genera known as classical producers of antibiotics were of high interest in this project. To increase the chances of discovering structurally new antibiotics, recent studies underlined the necessity to exploit new bacterial genera with genetic distance to the known compound producers. Finally, it was stated that chemical diversity depends on the genetic distance (Hoffmann et al., 2018).

Microbial strains isolated in this study were classified by rRNA gene amplification, subsequent Sanger sequencing, and BLASTn analysis of the sequencing results against public databases (see **Figure 9**). Phylogenetic analysis of the 16*S* rRNA gene is a standard procedure in microbiology for the identification of bacteria (Woese et al., 1987; Weisburg et al., 1991; Lane, 1991). Nevertheless, identification based on 16*S* rRNA gene homology is often not sufficient to distinguish between species or even genera (Fox et al., 1992; Vandamme et al., 1996). Due to these drawbacks, in this study best BLASTn hits of rRNA gene sequences were only used to scrutinise the phylogenetic background of the strains and to predict the putative closest relative within the database. Commonly used homology thresholds to strictly define species (e.g. ≤ 98% 16*S* rRNA gene homology) were left aside and assignments of species names and genera have to be considered putative for the time beeing.

Whole genome sequencing is the molecular method of choice to perform a more precise taxonomic identification (Poretsky et al., 2014). Three of the most promising strains from this study were therefore subjected to whole genome sequencing. One of these isolates was strain 39, which showed inhibitory effects against the Gram-negative *E. coli* (**Table 7**). The initial phylogenetic analysis *via* 16*S* rRNA gene sequencing and BLASTn analysis had identified *Arthrobacter sp*. as the closest relative with a sequence identity of 100%. The newly sequenced genome of strain 39 was compared to NCBI's "Reference Genomes" on the whole genome level. It was shown that the best match among the reference genomes was *Glutamicibacter arilaitensis*, but it has to be taken into account that the homologous region between both strains only comprises 12.8% of the genome sequences. While for this conserved DNA the overall sequence identity is 88.47%, the rest of the genomes have no significant similarities. Thus, the 16*S*-based classification and the whole-genome approach do not result in the same closest relative, which is not surprising, because the reference genome data base includes way less species than the database used for 16*S* BLAST analysis. Nevertheless *Arthrobacter* and *Glutamicibacter* are related species, since *Glutamicibacter* is a genus reclassified from *Arthrobacter* (Busse, 2016). However, a taxonomic classification of strain 39 on the genome level has to be postponed, due to the low amount of conserved DNA shared with so far sequenced and publically available bacterial genomes.

The genome of strain 39 comprises a small nonribosomal protein synthesis/polyketide synthesis (NRPS/PKS) biosynthetic gene cluster (BGC). Hence, further investigations on both the BGC as well as the strain itself will be necessary for phylogenetic classification of this strain and its antimicrobial compound.

The strains 2ABFH4 and Wild Mid 14 were previously regarded as unspecified isolates, based on 16*S* best BLASTn. The whole genome BLASTn of strain 2ABFH4 resulted in the identification of *Providencia rettgeri* as its closest related species. However, the genome of strain 2ABFH4 and *P. rettgeri* only share 3.92% of their genome. This rather small homologous region has a sequence identity of 86.5%. Due to these results, a phylogenetic identification is not possible for the time being. AntiSmash analyses were not able to detect any known BGC. Furthermore, a reclassification of that strain based on the bioinformatic analysis and compound isolation would certainly contribute to the identification of strain 2ABFH4 and its spectrum of antimicrobials produced. Finally, it cannot be excluded that isolate 2ABFH4 might also represent a new species.

In contrast, the isolate Wild Mid 14 shares 94% of its genomic sequence with 99.4% identity with *Proteus mirabilis*. Because of this high similarity, it can be assumed that this isolate is a member of the genus *Proteus.* Strain Wild Mid 14 is therefore the only one of all three whole genome sequenced isolates that can be attributed to a defined genus.

Aim 3: Analytic and isolation of interesting candidates

7.4 The isolated strain FF6-H_2O

Strain FF6-H_2O was isolated from the gut of the burying beetle by long-term cultivation on 1.5% water agar. The nutrient deficiency led to slow growth of the bacteria and, therefore, prevented bacterial generalists to overgrow other, more specialised, slow-growing taxa. This isolate was identified as *Serratia plymuthica* and exhibited no red pigmentation. Hence, the production of prodigiosin under the experimental conditions applied could be excluded. Moreover, the isolates FF6- H_2O and 56 were the only ones of the genus *Serratia* to display activity against all microbes tested. Because of this broad antimicrobial effect, LC/MS and MS/MS analyses were performed, and subsequently AntiBase 2014 was searched for hits as previously described. Finally, a literature search for broad antimicrobial activity produced by *Serratia* species led to the hypothesis of zeamine I ($C_{49}H_{101}N_4O_6$) (Masschelein et al., 2013). Zeamines are known as broad-spectrum antibiotics encoded by a hybrid NRPS/PKS biosynthetic gene cluster. Notably, they also exhibit nematicidal activity (Hellberg et al., 2015). The compound is targeting the cell wall unspecifically (Masschelein et al., 2015), thus explaining its broad antimicrobial activity. Further LC/MS experiments could not support the initial hypothesis. It was not possible to reconfirm a pesudomolecular ion of zeamine I as initially detected. Moreover, HPLC separation and fraction collection as well as subsequent bioactivity-guided testing revealed different bioactive fractions (**Figure 16**). This indicates either derivates of zeamine I e.g. bioactivty derivatives of the molecule, or an initial misidentification of the antimicrobially active compound as zeamine I. Therefore, FLASH chromatography followed by activity-guided screenings of the resulting fractions was performed multiple times, without a positve identification of a bioactive fraction. Additionally, different chemical extraction methods were evaluated for improving the compound isolation process. The following strategies were applied to obtain high amounts of crude extracts after fermentation: (i) liquid-liquid extraction with EtOAc, (ii) cultivation with the adsorbent XAD 2 and (iii) the classic freeze-drying approach as described above. Nevertheless, all extracts were devoid of bioactivity after large-scale fermentation. The use of *N*-(3-oxohexanoyl)-C6-homoserine lactone, a naturally occurring quorum sensing molecule, was not successful. Orininally, this molecule was described as an inducer of the zeamine I biosynthesis by *S. plymuthica* (Masschelein et al., 2013). Consequnetly, this negative result further supports the hypotheis of the presence of an antibiotic compound other than zeamine I.

As previously mentioned, the loss of bioactivity is a global phenomenon, the reasons for which can often not be identified. Often, co-cultivation experiments or the supplementation with cell-free supernatant of other bacterial cultures are considered. The presence of other bacteria or metabolites can induce the antimicrobial compound production and would also be appropriate for fermentation

scale-up. Cell-free *E. coli* supernatant was therefore tested for the induction of zeamine I biosynthesis; however this approach was also unsuccessful.

Whole genome sequencing is another possible method to uncover the antimicrobial potential of the strain FF6-H_2O. Generally, this approach facillitates a defined phylogenetic identification as well as an identification of BGC's by AntiSMASH. As previously shown in this study for isolate 39, 16*S* gene-based identification can be insufficient to identify a species. Therefore, the phylogenetic classification of the strain FF6-H_2O had to remain hypothetical. Consequnently, whole genome sequenzing or PCR trargeting significant features of BGCs can support the identification (Lemetre et al., 2017). Therefore, any specific approach towards an induction of the BGC is based on hypothesis.

7.5. The isolated compound serrawettin W2

Strain 2MH3-2 was identified as *S. marcescens* and exhibited antimicrobial activity against *S. aureus*. The HLPC separation and collection of the resulting fractions led to the identification of a medium-polar antimicrobial compound. Subsequent isolation by FLASH chromatography yielded 5 mg of pure compound derived from 5 L fermentation. The genus *Serratia*, like most of the other *Enterobacteriaceace*, grows fast to high cell density. Moreover, the production of extracellular proteins resulted in a high amount of crude extract. Hence, to reduce its amount, proteins had to be precipitated to prevent overloading of the FLASH column and to facilitate an optimal separation.

Further NMR analysis confirmed the production of the cyclic depsipeptide serrawettin W2, as previously hypothesised. Serrawettin W2 is a secondary metabolite produced by *S. marcescens* and has previously been reported as a bacterial surfactant (Matsuyama et al., 1986; Matsuyama et al., 1992). Bacterial surfactants, e.g. surfactin from *Bacillus subtilis*, are often lipopeptides (Raaijmakers et al., 2010), which are capable of lowering the surface tension. Thereby, the bacteria are able to spread on semisolid surfaces and therefore, these compounds are also called wetting agents (Matsuyama and Nakagawa, 1996). These biosurfactants are considered alternatives to other antimicrobial agents against different clinical infections (Fariq and Saeed, 2016). Serrawettin W2 has been tested for its antimicrobial activity and was able to suppress both Gram negative and -positive bacteria (Su et al., 2016). The latter findings are in contrast to the results of this project. Here, serrawettin W2 only inhibited Gram-positive bacteria such as *B. subtilis*, *L. monocytogenes* and *S. aureus* (MSSA and MRSA).

7.5.1 Ecological role of serrawettin W2

Insect-associated beneficial bacteria and their bioactive secondary metabolites have gained much interest of the scientific community. For a long time, the contribution of microbial symbionts to the biosynthesis of low-molecular-weight compounds was neglected although the metabolites themselves have been known for decades. For example, the biosynthesis of the antitumour amide pederin has been discovered in an unculturable symbiont of the *Paederus* beetle (Piel, 2002). The highly toxic pederin is used by the beetle for chemical defence (Dettner, 1987). Moreover, antibiotic-producing symbiontic *Streptomyces* species have been intensively studied (Kaltenpoth, 2009) and reported to be part of the evolutionary evolved defensive symbiosis with the European beewolf *Philantus triangulum* (Krois et al., 2010; Engl et al., 2018).

Due to these findings, it has been hypothesised that the preservation process of *N*. *vespilloides* is supported by beneficial bacteria producing bioactive low-molecular-weight compounds (Degenkolb et al., 2011). The recently published results of Shukla et al. (2018b) underline the ecological importance of the genus *Serratia*. Notably, a number of *Serratia* strains displaying antimicrobial bioactivities have been isolated and investigated in this thesis. The compound serrawettin W2 isolated from a *S. marcescens* strain displayed antimicrobial and nematostatic activities that were hypothesised to be of importance for carcass preservation. Additionally, serrawettin W2 has been previously reported as an efficient repellent against the nematode *Caenorhabditits elegans* by Pradel et al. (2007). Furthermore, the burying beetle *N*. *vespilloides* was found to be associated with parasitic nematodes (Richter, 1993). Recent studies investigated another parasitic nematode that resembles very much *Rhabditoides regina.* The authors stated a negative influence on carcass health, larval survival, and larval mass, respectively (Wang and Rozen, 2017; Wang and Rozen, 2018b). It is therefore conceivable that serrawettin W2 helps the beetle to control the nematode load on the carcass itself and in its immediate vicinity, as supported by the findings of this study. The inhibition of Gram-positive bacteria may also be beneficial for carcass sanitation and preservation.

Notably, this thesis describes for the first time a structurally defined, low-molecular-weight antimicrobial and nematostatic compound as a secondary metabolite of a gut symbiont of *N*. *vespilloides*. However, further investigations are required to link this compound to the process of carcass preservation and the unique ecology of *N. vespilloides*.

7.6 The influence of the microbial gut symbionts on *N. vespilloides* and its life cycle

The preservation process of the burying beetle is a poorly understood phenomenon, which still fascinates the scientific community. The antimicrobial effects, which are essential for preservation of a rich, but highly susceptible nutrient source, have to be exceptionally efficient on one hand, but must not harm the developement of larvae on the other. Therefore, the major question is whether the beetle itself, the gut microbiome, or both "components" synergistically preserve the carcass by the production of carcass-preserving and repellent agents.

To answer this question, the *N. vespilloides* was investigated for the expression and secretion of antimicrobial peptides (AMPs). Those have been investigated extensively in insects, and some show promising antimicrobial potential (Wiesner and Vilcinskas, 2010; Tonk and Vilcinskas, 2017). Twenty-seven AMPs and three lysozymes have been reported for *N. vespilloides*. When the beetle was exposed to carrion, both c-lysozyme-2 and thaumatin-4 were found to be highly expressed (Jacobs et al., 2016). Both of these antimicrobial proteins are also present in the anal secretion of the beetle. Nevertheless, it is hypothesized that the sanitation of the carcass requires a broad spectrum of antimicrobial compounds (Jacobs et al., 2016), including AMP's. Therefore, the target-oriented exploration of the gut microbiome might fill another gap in our current understanding of the preservation process. It has been previously shown that the burying beetle *N. vespilloides* hosts an unusual microbiome (Kaltenpoth and Steiger, 2014). Although the role of antimicrobial low-molecular-weight compounds was previously hypothesised (Degenkolb et al., 2011), the production of antibiotic or carcass-preserving compounds has not yet been linked to a spectrum of defined microbial producers. To date, it could only be shown that certain microbes such as *Wohlfartiimonas*, *Providencia*, *Morganella*, *Vagococcus*, *Myroides,* and the yeast *Yarrowia*, are transferred to the carcass. Notably, these microbes are neither present on untreated carrion nor in the soil (Shukla et al., 2018).

In this thesis, a cultivation-dependent approach has been used for the first time to investigate the antimicrobial potential of the *N. vespilloides* gut microbiome. To date, little is known if the antimicrobial potential observed in this project is of any relevance for the beetle. The multiple antimicrobial effects described in this study were observed under laboratory conditions, only. Thus, *in vivo* experiments are inevitably required to link those antimicrobial effects to the beetle's unique ecology.

Recently, co-cultivation experiments with *Morganella morganii* isolated from *N*. *vespilloides* gut and *Serratia marcescens* isolated from decomposing mice carcasses were conducted. Wang and Rozen (2018a) demonstrated the inhibition of their *S. marcescens* strain by the *M. morganii* isolated. In this study, the same authors isolated a red-pigmented *S. marcescens*, which corresponds to the production of prodigiosin (Hejazi and Falkiner, 1997). In contrast, the *Serratia* strains isolated in the project reported here, never exhibited any red pigmentation. This gives evidence to the phylogenetic and

chemical differences to the strain used by Wang and Rozen. The ecological relevance of the genus *Serratia* for *N. vespilloides* is unclear until now. Due to the result of *Serratia* being one of the most prominent genera on tended carcasses (Shukla et al., 2018b), it can be assumed that this genus and its secondary metabolites are of importance for carcass health and preservation. This also supports the high abundance of *Serratia* strains isolated in this project and their antimicrobial effects (**Figure 13**). Isolates FF6-H_2O and 56 displayed broad-spectrum inhibition of the pathogens tested whereas others e.g. isolate 2MH3-2 exclusively inhibited Gram-positives. Consequently, it can be stated that isolates of the genus *Serratia* are benefitial to the beetle`s ecology.

Perspective

The exceptional lifestyle of *N. vespilloides* has attracted the interest of scientist for centuries (Fabre, 1914; Pukowski, 1933). Recent contributions addressed the preservation strategy and the chemically diverse spectrum of antimicrobial compounds produced (Degenkolb et al., 2011; Jacobs et al., 2016; Vogel et al., 2017). The knowledge-based approach to explore this untapped resource for natural products resulted in the identification of numerous bacteria with antimicrobial activity. Beside those, several unspecified bacteria producing a broad spectrum of antimicrobial bioactivities were found. Some of those were chosen for whole genome sequencing and biosynthetic genecluster analysis to to gain more detailed insights into the antimicrobial potential of the gut microbiome. Furthermore, this project contributed to the understanding of the preservation process from the bacterial side.

Still, additional experiments and investigations are necessary to understand the preservation process of *N. vespilloides* in more detail. To access further bacterial gut symbionts, a variety of cultivation approaches with media designed to meet the physiological parameters and needs of the gut symbionts are required. This strategy may lead to the target-oriented discovery and cultivation of hitherto uncultivated microorganisms. The use of complex and minimal media without consideration of pH-value and oxygen conditions in the gut resulted in a rather undirected cultivation of microorganisms. Imitating the gut's physiological conditions could support the growth of more specialised, gut-adopted taxa. This may lead to the detection and production of a more diverse spectrum of bioactive secondary metabolites.

The primary screening (see **6.3.1 and Table S2**) revealed a variety of antimicrobial effects. Nevertheless, most of the Gram-negative inhibition was lost in the course of HPLC separation. Therefore, further experiments to trigger the induction of the desired, original bioactivity are required.A first possibility would be the co-cultivation with a microbial competitor to induce the natural defence mechanisms of the strain. Another option could include a variety of different cultivation media to screen for bioactivity induction within different nutrient settings.

Alternatively, cultivation and induction-independent approaches to broaden the antimicrobial potential of the gut microbiome should be promising. The expanded screening for BGCs based on full genome sequencing can be an appropriate but comparatively expensive option. Without the bioactivity-guided selection of strains, this could end up in high costs with rather low outcome. Moreover, such strategy is not suitable for a high-throughput screening.

Further on, strain 39 could not be identified as *Glutamicibacter* on whole genome level. Its NRPS/PKS BGC is intended for heterologous expression in a *Streptomyces* species. In parallel, classical compound isolation can be performed in case of activity loss after expression.

Future work should be aimed at the genome announcement of strain 39 and the bioactivity-guided identification of the antimicrobial compound produced by this strain. Also, strain 2ABFH4 is another possible candidate for genome announcement. With its homology of 3.9% shared with *Providencia rettgeri*, the possibility of discovering a new species is comparetively high.

To finally proof the ecologically relevance of serrawettin W2 for *N. vespilloides*, LC/MS analysis of the anal secretions can be conducted to search for the presence of this peptide. Also matrix-assisted laser desorption ionization imaging (MALDI) would be a state-of-the-art option to verify the production of serrawettin W2 by *S. marcescens* in the beetle's gut. This would further coroborate the discoveries made under laboratory conditions, thus confirming the observation that *N. vespilloides* is using both its own AMPs as well as antimicrobial compounds for carcass preservation.

Literature

AGOSTINI-COSTA, T. D. S., VIEIRA, R. F., BIZZO, H. R., SILVEIRA, D. & GIMENES, M. A. 2012. *Secondary Metabolites*, IntechOpen

AKMAN, L., YAMASHITA, A., WATANABE, H., OSHIMA, K., SHIBA, T., HATTORI, M. & AKSOY, S. 2002. Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genetics,* **32,** 402-407

ALBERT, M. J., ALAM, K., ISLAM, M., MONTANARO, J., RAHMAN, A. S. M. H., HAIDER, K., HOSSAIN, M. A., KIBRIYA, A. K. M. G. & TZIPORI, S. 1991. *Hafnia alvei*, a probable cause of diarrhea in humans. *Infection and Immunity,* **59,** 1507-1513

ALLEN, H. K., CLOUD-HANSEN, K. A., WOLINSKI, J. M., GUAN, C. H., GREENE, S., LU, S., BOEYINK, M., BRODERICK, N. A., RAFFA, K. F. & HANDELSMAN, J. 2009. Resident microbiota of the gypsy moth midgut harbors antibiotic resistance determinants. *DNA and Cell Biology,* **28,** 109-117

ALLEN, H. K., DONATO, J., WANG, H. H., CLOUD-HANSEN, K. A., DAVIES, J. & HANDELSMAN, J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology,* **8,** 251-259

ALLISON, C., LAI, H. C., GYGI, D. & HUGHES, C. 1993. Cell-differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemoattractant for swarming cells. *Molecular Microbiology,* **8,** 53-60

AMERICAN-ACADEMY-OF-MICROBIOLOGY 2009. Antibiotic Resistance: An ecological perspective on an old problem. *American Academy of Microbiology*

AUGUSTUS, A. M., CELAYA, T., HUSAIN, F., HUMBARD, M. & MISRA, R. 2004. Antibiotic-sensitive TolC mutants and their suppressors. *Journal of Bacteriology,* **186,** 1851-1860

BALTZ, R. H., 2007. Antimicrobials from actinomycetes: Back to the future. *Microbe*, **2**, 125-131

BALTZ, R. H. 2008. Renaissance in antibacterial discovery from actinomycetes. *Current Opinion in Pharmacology,* **8,** 557-563

BALTZ, R. H. 2014. MbtH homology codes to identify gifted microbes for genome mining. *Journal of Industrial Microbiology & Biotechnology,* **41,** 357-369

BALTZ, R. H. 2016. Genetic manipulation of secondary metabolite biosynthesis for improved production in *Streptomyces* and other actinomycetes. *Journal of Industrial Microbiology & Biotechnology,* **43,** 343-370

BENTLEY, S. D., CHATER, K. F., CERDENO-TARRAGA, A. M., CHALLIS, G. L., THOMSON, N. R., JAMES, K. D., HARRIS, D. E., QUAIL, M. A., KIESER, H., HARPER, D., BATEMAN, A., BROWN, S., CHANDRA, G., CHEN, C. W., COLLINS, M., CRONIN, A., FRASER, A., GOBLE, A., HIDALGO, J., HORNSBY, T., HOWARTH, S., HUANG, C. H., KIESER, T., LARKE, L., MURPHY, L., OLIVER, K., O'NEIL, S., RABBINOWITSCH, E., RAJANDREAM, M. A., RUTHERFORD, K., RUTTER, S., SEEGER, K., SAUNDERS, D., SHARP, S., SQUARES, R., SQUARES, S., TAYLOR, K., WARREN, T., WIETZORREK, A., WOODWARD, J., BARRELL, B. G., PARKHILL, J. & HOPWOOD, D. A. 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature,* **417,** 141-147

BERDELA, G., LUSTIGMAN, B. & SHUBECK, P. P. 1994. A list of bacterial-flora residing in the mid and hindgut regions of 6 species of carrion beetles (Coleoptera, Silphidae). *Entomological News,* **105,** 47-58

BÉRDY, J. 2005. Bioactive microbial metabolites. *The Journal of Antibiotics,* **58,** 1-26

BLIN, K., MEDEMA, M. H., KOTTMANN, R., LEE, S. Y. & WEBER, T. 2017. The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters. *Nucleic Acids Research,* **45,** D555-D559

BOCKER, H, 1978. Biometrische Versuchsplanung In: Rutloff, H.: Industrielle Enzyme . 1. Auflage, S. 142-153, VEB Fachbuchverlag Leipzig 1978

BODE, H. B., BETHE, B., HOFS, R. & ZEECK, A. 2002. Big effects from small changes: Possible ways to explore nature's chemical diversity. *Chembiochem,* **3,** 619-627

BOHNERT, J. A., SCHUSTER, S. & KERN, W. V. 2013. Pimozide inhibits the AcrAB-TolC efflux pump in *Escherichia coli*. *Open Microbiology Journal,* **7,** 83-86

BRODERICK, N. A. & LEMAITRE, B. 2012. Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes,* **3,** 307-321

BROOKE, J. S. 2012. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clinical Microbiology Reviews,* **25,** 2-41

BURGESS, J. G., JORDAN, E. M., BREGU, M., MEARNS-SPRAGG, A. & BOYD, K. G. 1999. Microbial antagonism: a neglected avenue of natural products research. *Journal of Biotechnology,* **70,** 27-32

BUSH, K., COURVALIN, P., DANTAS, G., DAVIES, J., EISENSTEIN, B., HUOVINEN, P., JACOBY, G. A., KISHONY, R., KREISWIRTH, B. N., KUTTER, E., LERNER, S. A., LEVY, S., LEWIS, K., LOMOVSKAYA, O., MILLER, J. H., MOBASHERY, S., PIDDOCK, L. J., PROJAN, S., THOMAS, C. M., TOMASZ, A., TULKENS, P. M., WALSH, T. R., WATSON, J. D., WITKOWSKI, J., WITTE, W., WRIGHT, G., YEH, P. & ZGURSKAYA, H. I. 2011. Tackling antibiotic resistance. *Nature Reviews Microbiology,* **9,** 894-896

BUSSE, H.-J. 2016. Review of the taxonomy of the genus *Arthrobacter*, emendation of the genus *Arthrobacter sensu lato*, proposal to reclassify selected species of the genus *Arthrobacter* in the novel genera *Glutamicibacter* gen. nov., *Paeniglutamicibacter* gen. nov., *Pseudoglutamicibacter* gen. nov., *Paenarthrobacter* gen. nov and *Pseudarthrobacter* gen. nov., and emended description of *Arthrobacter roseus*. *International Journal of Systematic and Evolutionary Microbiology,* **66,** 9-37

CAO, Z. J., ZHANG, Q., WEI, D. K., CHEN, L., WANG, J., ZHANG, X. Q. & ZHOU, M. H. 2009. Characterization of a novel *Stenotrophomonas* isolate with high keratinase activity and purification of the enzyme. *Journal of Industrial Microbiology and Biotechnology,* **36,** 181-188

CAVANAGH, J. P., WOLDEN, R., HEISE, P., ESAIASSEN, E., KLINGENBERG, C. & FREDHEIM, E. G. A. 2016. Antimicrobial susceptibility and body site distribution of community isolates of coagulase-negative staphylococci. *Apmis,* **124,** 973-978

CHALLINOR, V. L. & BODE, H. B. 2015. Bioactive natural products from novel microbial sources. *Annals of the New York Academy of Sciences,* **1354,** 82-97

CHANG, H. K. & ZYLSTRA, G. J. 2010. Xanthomonads. *In:* TIMMIS, K. N. (ed.) Handbook of hydrocarbon and lipid microbiology. Springer Berlin Heidelberg, pp. 1805-1811

CHAPMAN, A. 2009. Numbers of living species in Australia and the world. 2nd edition, Australian Government, Department of the Environment, Water, Heritage and the Arts

CHATER, K. F. & MERVYN, J. B. 1997. Regulation of bacterial antibiotic Production. In: REHM, H.-J., REED, G., PÜHLER, A., STADLER, P., Biotechnology 2nd, completely revised edition. Vol.7: Products of secondary metabolism (eds: KLEINKAUF, H., VON DÖHREN, H.) VCH Weinheim pp.55-105

CHIN, C. S., ALEXANDER, D. H., MARKS, P., KLAMMER, A. A., DRAKE, J., HEINER, C., CLUM, A., COPELAND, A., HUDDLESTON, J., EICHLER, E. E., TURNER, S. W. & KORLACH, J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nature Methods,* **10,** 563-569

CIMERMANCIC, P., MEDEMA, M. H., CLAESEN, J., KURITA, K., BROWN, L. C. W., MAVROMMATIS, K., PATI, A., GODFREY, P. A., KOEHRSEN, M., CLARDY, J., BIRREN, B. W., TAKANO, E., SALI, A., LININGTON, R. G. & FISCHBACH, M. A. 2014. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. *Cell,* **158,** 412-421

COETZEE, J. N. 1967. Bacteriocinogeny in strains of *Providence* and *Proteus morganii*. *Nature,* **213,** 614-616

COTTER, S. C. & KILNER, R. M. 2010. Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology,* **79,** 35-43

DARDIC, D., LAURO, G., BIFULCO, G., LABOUDIE, P., SAKHAII, P., BAUER, A., VILCINSKAS, A., HAMMANN, P. E. & PLAZA, A. 2017. Svetamycins A-G, unusual piperazic acid-containing peptides from *Streptomyces* sp. *Journal of Organic Chemestry,* **82,** 6032-6043

DAY, M. F. 1951. Studies on the digestion of wool by insects. I. Microscopy of digestion of wool by clothes moth larvae (*Tineola bisselliella* Humm). *Australian Journal of Scientific Research,* **4,** 42-48

DE VUYST, L., FOULQUIE MORENO, M. R. & REVETS, H. 2003. Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. *International Journal of Food Microbiology,* **84,** 299-318

DEGENKOLB, T., DÜRING, R. A. & VILCINSKAS, A. 2011. Secondary metabolites released by the burying beetle *Nicrophorus vespilloides*: chemical analyses and possible ecological functions. *Journal of Chemical Ecology,* **37,** 724-735

DEMAIN, A. L. 1998. Induction of microbial secondary metabolism. *International Microbiology,* **1,** 259-264

DETTNER, K., 1987, Chemosystematics and evolution of beetle chemical defenses. *Annual Review of Entomology*, **32**, 17-48

DROZDZYNSKA, A., PAWLICKA, J., KUBIAK, P., KOSMIDER, A., PRANKE, D., OLEJNIK-SCHMIDT, A. & CZACZYK, K. 2014. Conversion of glycerol to 1,3-propanediol by *Citrobacter freundii* and *Hafnia alvei* - newly isolated strains from the *Enterobacteriaceae*. *New Biotechnology,* **31,** 402-410

FABRE, J. H., Bilder aus der Insektenwelt. 1914 Erste bis vierte Reihe in zwei Bänden. Stuttgart: Kosmos, Franckh'sche Verlagshandlung, S. 14-28

FARIQ, A. & SAEED, A. 2016. Production and biomedical applications of probiotic biosurfactants. *Current Microbiology,* **72,** 489-495

FARROW, J. A. E., LAWSON, P. A., HIPPE, H., GAUGLITZ, U. & COLLINS, M. D. 1995. Phylogenetic evidence that the Gram-negative nonsporulating bacterium *Tissierella* (*Bacteroides*) *praeacuta* is a member of the *Clostridium* subphylum of the Gram-positive bacteria and description of *Tissierella creatinini* sp. nov. *International Journal of Systematic Bacteriology,* **45,** 436-440

FEHER, M. & SCHMIDT, J. M. 2003. Property distributions: Differences between drugs, natural products, and molecules from combinatorial chemistry. *Journal of Chemical Information and Computer Sciences,* **43,** 218-227

FICKERS, P. 2012. Antibiotic compounds from *Bacillus*: Why are they so amazing? . *American Journal of Biochemistry and Biotechnology* **8,** 38-43

FICKERS, P., BENETTI, P. H., WACHE, Y., MARTY, A., MAUERSBERGER, S., SMIT, M. S. & NICAUD, J. M. 2005. Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. *FEMS Yeast Research,* **5,** 527-543

FLEMING, A. 1929. On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenzae*. 1929. *The British Journal of Experimental Pathology,* **79,** 780-790

FLYG, C., KENNE, K. & BOMAN, H. G. 1980. Insect pathogenic properties of *Serratia marcescens* - phage-resistant mutants with a decreased resistance to *Cecropia* immunity and a decreased virulence to *Drosophila*. *Journal of General Microbiology,* **120,** 173-181

FOX, G. E., J. D. WISOTZKEY, JURTSHUK P. 1992. How close is close: 16*S* rRNA sequence identity may not be sufficient to guarantee species identity. *International Journal of Systematic Bacteriology*. **42**, 166–170

GALAC, M. R. & LAZZARO, B. P. 2011. Comparative pathology of bacteria in the genus *Providencia* to a natural host, *Drosophila melanogaster*. *Microbes and Infection,* **13,** 673-683

GERC, A. J., SONG, L. J., CHALLIS, G. L., STANLEY-WALL, N. R. & COULTHURST, S. J. 2012. The insect pathogen *Serratia marcescens* Db10 uses a hybrid non-ribosomal peptide synthetase-polyketide synthase to produce the antibiotic althiomycin. *PLoS ONE,* **7**, e44673

GILBERT, E. J. 1993. *Pseudomonas* lipases - biochemical-properties and molecular-cloning. *Enzyme and Microbial Technology,* **15,** 634-645

GOLDMAN, M. L. & RAYMAN, M. M. 1952. Hydrolysis of fats by bacteria of the *Pseudomon* as genus. *Food Research,* **17,** 326-337

GRIMONT, P. A. & GRIMONT, F. 1978. The genus *Serratia*. *Annual Review of Microbiology,* **32,** 221-248

GROOMBRIDGE, B. A. J., MD 2002. World atlas of biodiversity. *University of California Press.*

GUERRERO, G., PERALTA, H., AGUILAR, A., DIAZ, R., VILLALOBOS, M. A., MEDRANO-SOTO, A. & MORA, J. 2005. Evolutionary, structural and functional relationships revealed by comparative analysis of syntenic genes in *Rhizobiales*. *BMC Evolutionary Biology,* **5**, 55

GUNTHARD, H. & PENNEKAMP, A. 1996. Clinical significance of extraintestinal *Hafnia alvei* isolates from 61 patients and review of the literature. *Clinical Infectious Diseases,* **22,** 1040-1045

HALL, C. L., WADSWORTH, N. K., HOWARD, D. R., JENNINGS, E. M., FARRELL, L. D., MAGNUSON, T. S. & SMITH, R. J. 2011. Inhibition of microorganisms on a carrion breeding resource: The antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environmental Entomology,* **40,** 669-678

HAMILTON, G. R. & BASKETT, T. F. 2000. In the arms of Morpheus: the development of morphine for postoperative pain relief. *Canadian Journal of Anaesthesia-Journal Canadien D Anesthesie,* **47,** 367-374

HEAD, I. M., SAUNDERS, J. R. & PICKUP, R. W. 1998. Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. *Microbial Ecology,* **35,** 1-21

HEJAZI, A. & FALKINER, F. R. 1997. *Serratia marcescens*. *Journal of Medical Microbiology,* **46,** 903-912

HELLBERG, J. E. E. U., MATILLA, M. A. & SALMOND, G. P. C. 2015. The broad-spectrum antibiotic, zeamine, kills the nematode worm *Caenorhabditis elegans*. *Frontiers in Microbiology,* **6**, 137

HOENIGER, J. F. 1964. Cellular changes accompanying the swarming of *Proteus mirabilis*. I. Observations of living cultures. *Canadien Journal of Microbiology,* **10,** 1-9

HOFFMANN, T., KRUG, D., BOZKURT, N., DUDDELA, S., JANSEN, R., GARCIA, R., GERTH, K., STEINMETZ, H. & MULLER, R. 2018. Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nature Communications,* **9**, 803

HOPWOOD, D. A. 2007. How do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? *Molecular Microbiology,* **63,** 937-940

HOUBRAKEN, J., FRISVAD, C., & SAMSON, R. A. 2011. Fleming's penicillin producing strain is not *Penicillium chrysogenum* but *P. rubens*. *IMA Fungus*, **2**, 87–95

HOYLES, L., LAWSON, P. A., FOSTER, C., FALSEN, E., OHLEN, M., GRAINGER, J. M. & COLLINS, M. D. 2000. *Vagococcus fessus* sp nov., isolated from a seal and a harbour porpoise. *International Journal of Systematic and Evolutionary Microbiology,* **50,** 1151-1154

HUNT, M., DE SILVA, N., OTTO, T. D., PARKHILL, J., KEANE, J. A. & HARRIS, S. R. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. *Genome Biology,* **16**, 294

JACOBS, C. G., STEIGER, S., HECKEL, D. G., WIELSCH, N., VILCINSKAS, A. & VOGEL, H. 2016. Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. *Scientific Reports,* **6,** 25409

JACOBS, C. G. C., WANG, Y., VOGEL, H., VILCINSKAS, A., VAN DER ZEE, M. & ROZEN, D. E. 2014. Egg survival is reduced by grave-soil microbes in the carrion beetle, *Nicrophorus vespilloides*. *BMC Evolutionary Biology,* **14**, 208

JANDA, J. M. & ABBOTT, S. L. 2006. The genus *Hafnia*: from soup to nuts. *Clinical Microbiology Reviews,* **19,** 12-28

JANDA, J. M. & ABBOTT, S. L. 2007. 16*S* rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of Clinical Microbiology,* **45,** 2761-2764

KALINOVÁ, B., PODSKALSKÁ, H., RŮŽIČKA, J. & HOSKOVEC, M. 2009. Irresistible bouquet of death-how are burying beetles (Coleoptera: Silphidae: *Nicrophorus*) attracted by carcasses. *Naturwissenschaften,* **96,** 889-899

KALTENPOTH, M., 2009, Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology*, **12**, 529-535

KALTENPOTH, M. & STEIGER, S. 2014. Unearthing carrion beetles' microbiome: characterization of bacterial and fungal hindgut communities across the Silphidae. *Molecular Ecology,* **23,** 1251-1267

KATZ, L. & BALTZ, R. H. 2016. Natural product discovery: past, present, and future. *Journal of Industrial Microbiology and Biotechnology,* **43,** 155-176

KILLER, J., ŠVEC, P., SEDLÁČEK, I., ČERNOHLÁVKOVÁ, J., BENADA, O., HRONCOVÁ, Z., HAVLÍK, J., VLKOVÁ, E., RADA, V., KOPEČNÝ, J. & KOFROŇOVÁ, O. 2014. *Vagococcus entomophilus* sp nov.,

from the digestive tract of a wasp (*Vespula vulgaris*). *International Journal of Systematic and Evolutionary Microbiology,* **64,** 731-737

KNIGHT, V., SANGLIER, J. J., DITULLIO, D., BRACCILI, S., BONNER, P., WATERS, J., HUGHES, D. & ZHANG, L. 2003. Diversifying microbial natural products for drug discovery. *Applied Microbiology and Biotechnology,* **62,** 446-458

KORONAKIS, V., ESWARAN, J. & HUGHES, C. 2004. Structure and function of TolC: the bacterial exit duct for proteins and drugs. *Annual Review of Biochemistry,* **73,** 467-489

KRISHNAN, M., BHARATHIRAJA, C., PANDIARAJAN, J., PRASANNA, V. A., RAJENDHRAN, J. & GUNASEKARAN, P. 2014. Insect gut microbiome - An unexploited reserve for biotechnological application. *Asian-Pacific Journal of Tropical Biomedicine,* **4,** 16-21

Kroiss, J., Kaltenpoth, M., Schneider, B., Schwinger, M. G., Hertweck, C., Maddula, R. K., Strohm, E., Svatos, A., 2010, Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring.*Nature Chemical Biology*, **6**, 261–263

LANE, D. J. 1991. 16S/23*S* rRNA sequencing. *In:* E. STACKEBRANDT, M. G. (ed.) *Nucleic Acid Techniques in Bacterial Systematics (Modern Microbiological Methods).* New York 1991. John Wiley & Sons. : Wiley-VCH.

LAPENDA, J. C., SILVA, P. A., VICALVI, M. C., SENA, K. X. F. R. & NASCIMENTO, S. C. 2015. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. *World Journal of Microbiology & Biotechnology,* **31,** 399-406

LEE, R. E., JR., ELNITSKY, M. A., RINEHART, J. P., HAYWARD, S. A., SANDRO, L. H. & DENLINGER, D. L. 2006. Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. *Journal of Experimental Biology,* **209,** 399-406

LEMETRE, C., MANIKO, J., CHARLOP-POWERS, Z., SPARROW, B., LOWE, A. J., BRADY; S. F., 2017, Bacterial natural product biosynthetic domain composition in soil correlates with changes in latitude on a continent-wide scale. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, 11615-11620

LEWIS, K. 2012. Antibiotics: Recover the lost art of drug discovery. *Nature,* **485,** 439-440

LEWIS, K., EPSTEIN, S., D'ONOFRIO, A. & LING, L. L. 2010. Uncultured microorganisms as a source of secondary metabolites. *Journal of Antibiotics,* **63,** 468-476

LI, J. W. & VEDERAS, J. C. 2009. Drug discovery and natural products: end of an era or an endless frontier? *Science,* **325,** 161-165

MAHLEN, S. D. 2011. *Serratia* infections: from military experiments to current practice. *Clinical Microbiology Reviews,* **24,** 755-791

MAPLESTONE, R. A., STONE, M. J. & WILLIAMS, D. H. 1992. The evolutionary role of secondary metabolites--a review. *Gene,* **115,** 151-157

MARTÍNEZ, B., RODRÍGUEZ, A. & SUÁREZ, E. 2016. Antimicrobial peptides produced by bacteria: The bacteriocins. *In:* VILLA, T. G. & VINAS, M. (eds.) *New Weapons to Control Bacterial Growth.* Cham: Springer International Publishing, pp. 15-26

MASSCHELEIN, J., CLAUWERS, C., STALMANS, K., NUYTS, K., DE BORGGRAEVE, W., BRIERS, Y., AERTSEN, A., MICHIELS, C. W. & LAVIGNEA, R. 2015. The zeamine antibiotics affect the integrity of bacterial membranes. *Applied and Environmental Microbiology,* **81,** 1139-1146

MASSCHELEIN, J., MATTHEUS, W., GAO, L. J., MOONS, P., VAN HOUDT, R., UYTTERHOEVEN, B., LAMBERIGTS, C., LESCRINIER, E., ROZENSKI, J., HERDEWIJN, P., AERTSEN, A., MICHIELS, C. & LAVIGNE, R. 2013. A PKS/NRPS/FAS hybrid gene cluster from *Serratia plymuthica* RVH1 encoding the biosynthesis of three broad spectrum, zeamine-related antibiotics. *PLoS ONE,* **8**, e54143

MATSUYAMA, T., KANEDA, K., NAKAGAWA, Y., ISA, K., HARAHOTTA, H. & YANO, I. 1992. A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and flagellum-independent spreading growth of *Serratia-marcescens*. *Journal of Bacteriology,* **174,** 1769-1776

MATSUYAMA, T., MURAKAMI, T., FUJITA, M., FUJITA, S. & YANO, I. 1986. Extracellular vesicle formation and biosurfactant production by *Serratia marcescens*. *Journal of General Microbiology,* **132,** 865-875

MATSUYAMA, T. & NAKAGAWA, Y. 1996. Bacterial wetting agents working in colonization of bacteria on surface environments. *Colloids and Surfaces B: Biointerfaces,* **7,** 207-214

MCMANUS, P. S., STOCKWELL, V. O., SUNDIN, G. W., JONES, A. L., Antibiotic use in plant agriculture. *Annual Review of Phytopathology*, **40**, 443-465

MEARNS-SPRAGG, A., BREGU, M., BOYD, K. G. & BURGESS, J. G. 1998. Cross-species induction and enhancement of antimicrobial activity produced by epibiotic bacteria from marine algae and invertebrates, after exposure to terrestrial bacteria. *Letters in Applied Microbiology,* **27,** 142-146

MILNE, L. J. & MILNE, M. 1976. Social-behavior of burying beetles. *Scientific American,* **235,** 84-89.

MITSCHER, L. A. & BAKER, W. 1998. Tuberculosis: a search for novel therapy starting with natural products. *Medical Research Reviews,* **18,** 363-374

MOBLEY, H. L. T. & BELAS, R. 1995. Swarming and pathogenicity of *Proteus mirabilis* in the urinary-tract. *Trends in Microbiology,* **3,** 280-284

MOORE, E. R. B., MAU, M., ARNSCHEIDT, A., BÖTTGER, E. C., HUTSON, R. A., COLLINS, M. D., VANDEPEER, Y., DE WACHTER, R. & TIMMIS, K. N. 1996. The determination and comparison of the 16*S* rRNA gene sequences of species of the genus *Pseudomonas* (*sensu stricto*) and estimation of the natural intrageneric relationships. *Systematic and Applied Microbiology,* **19,** 478-492

NAVRE, M. 1998. Application of combinatorial chemistry to antimicrobial drug discovery. *Expertert Opinion on Investigational Drugs,* **7,** 1257-1271

NEWMAN, D. J. & CRAGG, G. M. 2007. Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products,* **70,** 461-477

NICHOLS, D., CAHOON, N., TRAKHTENBERG, E. M., PHAM, L., MEHTA, A., BELANGER, A., KANIGAN, T., LEWIS, K. & EPSTEIN, S. S. 2010. Use of Ichip for high-throughput in situ cultivation of "uncultivable" microbial species. *Applied and Environmental Microbiology,* **76,** 2445-2450

O'DONNELL, K. 1993. *Fusarium* and its near relatives. *In:* REYNOLDS, D. R. & TAYLOR, J. W. (eds.) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics : proceedings of an international symposium, Newport, Oregon, 4-7 August 1992. CAB International, pp. 225-236

ONAKA, H., MORI, Y., IGARASHI, Y. & FURUMAI, T. 2011. Mycolic acid-containing bacteria induce natural-product biosynthesis in Streptomyces species. *Applied and Environmental Microbiology,* **77,** 400-406

PACKEISER, H., LIM, C., BALAGURUNATHAN, B., WU, J. & ZHAO, H. 2013. An extremely simple and effective colony PCR procedure for bacteria, yeasts, and microalgae. *Applied Biochemestry and Biotechnology,* **169,** 695-700

PAGES, J. M., JAMES, C. E. & WINTERHALTER, M. 2008. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nature Reviews Microbiology,* **6,** 893-903

PARK, M., KIM, C., YANG, J., LEE, H., SHIN, W., KIM, S. & SA, T. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiological Research,* **160,** 127-133

PARKER, W. L., RATHNUM, M. L., WELLS, J. S., TREJO, W. H., PRINCIPE, P. A. & SYKES, R. B. 1982. Sq 27,860, a simple carbapenem produced by species of *Serratia* and *Erwinia*. *Journal of Antibiotics,* **35,** 653-660

PETTIT, R. K. 2009. Mixed fermentation for natural product drug discovery. *Applied Microbiology and Biotechnology,* **83,** 19-25

PHAM, V. H. T. & KIM, J. 2012. Cultivation of unculturable soil bacteria. *Trends in Biotechnology,* **30,** 475-484

PIEL, J. 2002 A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of Paederus beetles. *Proceedings of the National Academy of Sciences of the United States of America*, **99**,14002-14007

PIEL, J. 2004. Metabolites from symbiotic bacteria. *Natural Product Reports,* **21,** 519-538

PIEL, J. 2011. Approaches to capturing and designing biologically active small molecules produced by uncultured microbes. *Annual Review of Microbiology,* **65,** 431-453

PORETSKY, R., RODRIGUEZ-R, LM., LUO, C., TSEMENTZI, D., KONSTANTINIDIS, KT. 2014. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. *PLoS ONE*, **9**, e93827

POTTER, R. F., D'SOUZA, A. W. & DANTAS, G. 2016. The rapid spread of carbapenem-resistant Enterobacteriaceae. *Drug Resistance Updates,* **29,** 30-46

PRADEL, E., ZHANG, Y., PUJOL, N., MATSUYAMA, T., BARGMANN, C. I. & EWBANK, J. J. 2007. Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences of the United States of America,* **104,** 2295-2300

PUKOWSKI, E. 1933. Ökologische untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* **27,** 518-586

RAAIJMAKERS, J. M., DE BRUIJN, I., NYBROE, O. & ONGENA, M. 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiology Reviews,* **34,** 1037-1062

RAJAGOPAL, R. 2009. Beneficial interactions between insects and gut bacteria. *Indian Journal of Microbiology,* **49,** 114-119

RAMOS, A. & DAMASO, D. 2000. Extraintestinal infection due to *Hafnia alvei*. *European Journal of Clinical Microbiology & Infectious Diseases,* **19,** 708-710

RAWLS, W. J., PACHEPSKY, Y. A., RITCHIE, J. C., SOBECKI, T. M. & BLOODWORTH, H. 2003. Effect of soil organic carbon on soil water retention. *Geoderma,* **116,** 61-76

RAZUMOV, A. S. 1932, The direct method of calculation of bacteria in water: Comparison with the Koch method. Mikrobiologija, **1**, 131-146

RICHTER, S. 1993. Phoretic association between the dauerjuveniles of *Rhabditis stammeri* (Rhabditidae) and life-history stages of the burying beetle *Nicrophorus vespilloides* (Coleoptera, Silphidae). *Nematologica,* **39,** 346-355

RYAN, R. P., MONCHY, S., CARDINALE, M., TAGHAVI, S., CROSSMAN, L., AVISON, M. B., BERG, G., VAN DER LELIE, D. & DOW, J. M. 2009. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology,* **7,** 514-525

SCHÄBERLE, T. F. & HACK, I. M. 2014. Overcoming the current deadlock in antibiotic research. *Trends in Microbiology,* **22,** 165-167

SCHERLACH, K. & HERTWECK, C. 2009. Triggering cryptic natural product biosynthesis in microorganisms. *Organic & Biomolecular Chemistry,* **7,** 1753-1760

SCHMIDTKE, L. M. & CARSON, J. 1994. Characteristics of *Vagococcus salmoninarum* isolated from diseased salmonid fish. *Journal of Applied Bacteriology,* **77,** 229-236

SCOTT, M. P. 1998. The ecology and behavior of burying beetles. *Annual Review of Entomology,* **43,** 595-618

SEEMANN, T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics,* **30,** 2068-2069

SHEKH, R. M. & ROY, U. 2012. Biochemical characterization of an anti-*Candida* factor produced by *Enterococcus faecalis*. *BMC Microbiology,* **12,** 132

SHEPPARD, D. C., TOMBERLIN, J. K., JOYCE, J. A., KISER, B. C. & SUMNER, S. M. 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology,* **39,** 695-698

SHERWOOD, V. 1996. Most heat tolerant. *Book of Insect Records.* University of Florida, Gaineswille: Department of Entomology & Nematology

SHUKLA, S. P., VOGEL, H., HECKEL, D. G., VILCINSKAS, A. & KALTENPOTH, M. 2018a. Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology,* **27,** 1980-1991

SHUKLA, S. P., PLATA, C., REICHELT, M., STEIGER S., HECKEL, D. G., KALTENPOTH, M., VILCINSKAS, A., VOGEL, H., 2018b, Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, 11274-11279

SIKES, D. S., VAMOSI, S. M., TRUMBO, S. T., RICKETTS, M. & VENABLES, C. 2008. Molecular systematics and biogeography of *Nicrophorus* in part – The *investigator* species group (Coleoptera : Silphidae) using mixture model MCMC. *Molecular Phylogenetics and Evolution,* **48,** 646-666

SOARES, J. 2011. The Nagoya Protocol and natural product-based research. *ACS Chemical Biology,* **6,** 289

SOLTER, L. F., LUSTIGMAN, B. & SHUBECK, P. 1989. Survey of medically important true bacteria found associated with carrion beetles (Coleoptera, Silphidae). *Journal of Medical Entomology,* **26,** 354-359

STACKEBRANDT, E. & GOEBEL, B. M. 1994. Taxonomic note: A place for DNA-DNA reassociation and 16*S* rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic and Evolutionary Microbiology,* **44,** 846-849

STALEY, J. T. & KONOPKA, A. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review ofMicrobiology,* **39,** 321-46

STOCK, I. & WIEDEMANN, B. 1998. Natural antibiotic susceptibility of *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens* and *P. rustigianii* strains. *Journal of Medical Microbiology,* **47,** 629-642

STURGILL, G. & RATHER, P. N. 2004. Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Molecular Microbiology,* **51,** 437-446

SU, C., XIANG, Z. J., LIU, Y. B., ZHAO, X. Q., SUN, Y., LI, Z., LI, L. J., CHANG, F., CHEN, T. J., WEN, X. R., ZHOU, Y. D. & ZHAO, F. R. 2016. Analysis of the genomic sequences and metabolites of *Serratia surfactantfaciens* sp. nov. YD25(T) that simultaneously produces prodigiosin and serrawettin W2. *BMC Genomics,* **17**, 865

THAKER, M. N., WANG, W., SPANOGIANNOPOULOS, P., WAGLECHNER, N., KING, A. M., MEDINA, R. & WRIGHT, G. D. 2013. Identifying producers of antibacterial compounds by screening for antibiotic resistance. *Nature Biotechnology,* **31,** 922-927

THOMSON, N. R., CROW, M. A., MCGOWAN, S. J., COX, A. & SALMOND, G. P. C. 2000. Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Molecular Microbiology,* **36,** 539-556

TONK, M. & VILCINSKAS, A. 2017. The Medical Potential of Antimicrobial Peptides from Insects. *Current Topics in Medicinal Chemistry,* **17,** 554-575

UNIPROT-CONSORTIUM 2018. UniProt: the universal protein knowledgebase (vol 45, pg D158, 2017). *Nucleic Acids Research,* **46,** 2699-2699

VANDAMME, P., POT, B., GILLIS,M., DE VOS, P., KERSTERS, K., SWINGS, J. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiology and Molecular Biology Reviews*. **60** 407-438

VOGEL, H., SHUKLA, S. P., ENGL, T., WEISS, B., FISCHER, R., STEIGER, S., HECKEL, D. G., KALTENPOTH, M. & VILCINSKAS, A. 2017. The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. *Nature Communications,* **8,** 15186

VON HOERMANN, C., STEIGER, S., MULLER, J. K. & AYASSE, M. 2013. Too fresh is unattractive! The attraction of newly emerged *Nicrophorus vespilloides* females to odour bouquets of large cadavers at various stages of decomposition. *PLoS ONE,* 8, e58524

WAKSMAN, S. A. & WOODRUFF, H. B. 1940. The soil as a source of microorganisms antagonistic to disease-producing bacteria. *Journal of Bacteriology,* **40,** 581-600

WANG, Y. & ROZEN, D. E. 2017. Fitness effects and transmission of phoretic nematodes of the burying beetle, *Nicrophorus vespilloides*. *bioRxiv*

WANG, Y. & ROZEN, D. 2018a. Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution,* **8,** 1646-1654

WANG, Y. & ROZEN, D. E. 2018b Fitness costs ofphoretic nematodes in the burying beetle, *Nicrophorus vespilloides*. *Ecololgy and Evolution*. 2018;00:1-10

WARNECKE, F., LUGINBUHL, P., IVANOVA, N., GHASSEMIAN, M., RICHARDSON, T. H., STEGE, J. T., CAYOUETTE, M., MCHARDY, A. C., DJORDJEVIC, G., ABOUSHADI, N., SOREK, R., TRINGE, S. G., PODAR, M., MARTIN, H. G., KUNIN, V., DALEVI, D., MADEJSKA, J., KIRTON, E., PLATT, D., SZETO, E., SALAMOV, A., BARRY, K., MIKHAILOVA, N., KYRPIDES, N. C., MATSON, E. G., OTTESEN, E. A., ZHANG, X. N., HERNANDEZ, M., MURILLO, C., ACOSTA, L. G., RIGOUTSOS, I., TAMAYO, G., GREEN, B. D., CHANG, C., RUBIN, E. M., MATHUR, E. J., ROBERTSON, D. E., HUGENHOLTZ, P. & LEADBETTER, J. R. 2007. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature,* **450,** 560-565

WATVE, M. G., TICKOO, R., JOG, M. M. & BHOLE, B. D. 2001. How many antibiotics are produced by the genus *Streptomyces*? *Archives of Microbiology,* **176,** 386-390

WEISBURG, W. G. BARNS, S. M., PELLETIER, D. A., LANE, D. J. 1991 16*S* ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*. **173**, 697-703

WHITE, T. J., BRUNS, T., LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A guide to methods and applications.*. San Diego: Academic Press., pp.315-322

WIESNER, J. & VILCINSKAS, A. 2010. Antimicrobial peptides The ancient arm of the human immune system. *Virulence,* **1,** 440-464

WILLIAMS, R. P. 1973. Biosynthesis of prodigiosin, a secondary metabolite of *Serratia-marcescens*. *Applied Microbiology,* **25,** 396-402

WITTE, W., 1998, Medical consequences of antibiotic use in agriculture. *Science*, **5353**, 996-997

WOESE, C. R. 1987. Bacterial evolution. *Microbiology Reviews*, **51**, 221–271

WOHLLEBEN, W., MAST, Y., STEGMANN, E. & ZIEMERT, N. 2016. Antibiotic drug discovery. *Microbial Biotechnology,* 9**,** 541-8

WUNDERLICHOVÁ, L., BUŇKOVÁ, L., KOUTNÝ, M., JANČOVÁ, P. & BUŇKA, F. 2014. Formation, degradation, and detoxification of putrescine by foodborne bacteria: A review. *Comprehensive Reviews in Food Science and Food Safety,* **13,** 1012-1030

ZERIKLY, M. & CHALLIS, G. L. 2009. Strategies for the discovery of new natural products by genome mining. *Chembiochem,* **10,** 625-633

ZHENG, W., ZHANG, Y., LU, H. M., LI, D. T., ZHANG, Z. L., TANG, Z. X. & SHI, L. E. 2015. Antimicrobial activity and safety evaluation of *Enterococcus faecium* KQ 2.6 isolated from peacock feces. *BMC Biotechnology,* **15,** 30

Appendix

Table S1: List of Isolates from the gut of *N. vespilloides*

Abbreviations used:

1. Capital letter: M= male
   F= female
2. Capital letter: F= forgut
   M= midgut
   H= hindgut

Wild = wild-living species
Faek= Fecal isolate
a= anaerobic cultivated
AB= agar supplemented with antibiotics
Man= agar supplemented with mannitol
H_2O= isolated from water agar after long-time cultivation
BHI/YGC/PGS= sued media for cultivation

Strain ID	**Best BLASTn: closest genetic relative available**
70	*Achromobacter piechaudii* strain Na28 16S ribosomal RNA gene, partial sequence
45	*Acinetobacter guillouiae* isolate OTU-b62 16S ribosomal RNA gene, partial sequence
39	*Arthrobacter* sp. GH01 16S ribosomal RNA gene, partial sequence
40	*Arthrobacter* sp. GH01 16S ribosomal RNA gene, partial sequence
YGC-10	*Candida ghanaensis* small subunit ribosomal RNA gene, complete sequence
Man._F._mid._1-2	*Carnobacterium maltaromaticum* strain BSS448 16S ribosomal RNA gene, partial sequence
Man._F._mid._2	*Carnobacterium maltaromaticum* strain BSS448 16S ribosomal RNA gene, partial sequence
Man._F._hind._2	*Carnobacterium maltaromaticum* strain Fish 09690 16S ribosomal RNA gene, partial sequence
a-7	*Carnobacterium maltaromaticum* strain Fish 11.890 A2 K 12 16S ribosomal RNA gene, partial sequence
Man._F._mid._1-1	*Carnobacterium maltaromaticum* strain Fish 11.890 A2 K 12 16S ribosomal RNA gene, partial sequence
Man._F._mid._4-1	*Carnobacterium maltaromaticum* strain Fish 11.890 A2 K 12 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
Man._F._mid._4-2	*Carnobacterium maltaromaticum* strain G117 16S ribosomal RNA gene, partial sequence
76	*Carnobacterium sp.* ES-11 16S rRNA gene, strain ES-11
a-1	*Carnobacterium sp*. K3 16S ribosomal RNA gene, partial sequence
a-8	*Carnobacterium sp*. K3 16S ribosomal RNA gene, partial sequence
Man._F._hind._1-1	*Carnobacterium sp.* K3 16S ribosomal RNA gene, partial sequence
Mannitol_MF3	*Carnobacterium sp*. K3 16S ribosomal RNA gene, partial sequence
AB2	*Chryseobacterium meningosepticum* HO1J100 16S ribosomal RNA gene, partial sequence
68	*Chryseobacterium sp*. T86F.09.P.MUS.NO.H.Kidney.D.M 16S ribosomal RNA gene, complete sequence
AB1	*Chryseobacterium sp*. T86F.09.P.MUS.NO.H.Kidney.D.M 16S ribosomal RNA gene, complete sequence
13	*Citrobacter koseri* partial 16S rRNA gene, isolate 248
95	*Enterobacter asburiae* partial 16S rRNA gene, strain LH74
1MM3	*Enterobacter asburiae* partial 16S rRNA gene, strain LH74
a-10	*Enterobacter asburiae* partial 16S rRNA gene, strain LH74
a-9	*Enterobacter cloacae* strain B9 16S ribosomal RNA gene, partial sequence
Faek1-2	*Enterobacteriaceae bacterium* ENUB8 16S ribosomal RNA genes, partial sequence
Faek3_BHI	*Enterobacteriaceae bacterium* ENUB8 16S ribosomal RNA genes, partial sequence
1FF2	*Enterobacteriaceae bacterium* Pokym2-a 16S ribosomal RNA genes, partial sequence
89	*Enterococcus avium* gene for 16S ribosomal RNA, partial sequence, isolate: 3-1-22
38	*Enterococcus durans* strain ChOPR-I-str31 16S ribosomal RNA gene, partial sequence
Faek_1-1	*Enterococcus faecalis* strain OGR1 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
2FM6_PGS	*Enterococcus malodoratus* gene for 16S ribosomal RNA, partial sequence, strain: JCM 8730
2FH5_PGS	*Enterococcus malodoratus* strain NBRC 100489 16S ribosomal RNA gene
3FA2	*Enterococcus moraviensis* strain NBRC 100710
3MA5	*Enterococcus phoeniculicola* strain NBRC 100711
2MF6_PGS	*Enterococcus raffinosus* gene for 16S ribosomal RNA, partial sequence, strain: JCM 8733
42	*Enterococcus sp*. INBio_4507C 16S ribosomal RNA gene, partial seq.
FF7_H20_BHI	*Erwinia amylovora* strain HSA 6 16S ribosomal RNA gene, partial seq.
YGC-12	*Galactomyces geotrichum* strain LMA-15 18S ribosomal RNA gene
2FH1_PGS	*Hafnia alvei* FB1, complete genome
2FH2_PGS	*Hafnia alvei* FB1, complete genome
2FH2-1_PGS	*Hafnia alvei* FB1, complete genome
36	*Hafnia alvei* partial 16S rRNA gene, isolate 212
82	*Hafnia alvei* partial 16S rRNA gene, isolate 212
2MF5_PGS	*Hafnia alvei* partial 16S rRNA gene, isolate 212
24	*Hafnia alvei* strain FDAARGOS_158, complete genome
2FM5_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
2MF2_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
2MF3_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
2MM1_BHI	*Hafnia alvei* strain FDAARGOS_158, complete genome
FH6_BHI	*Hafnia alvei* strain FDAARGOS_158, complete genome
FM1_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
MF3_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
MF5_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
MH2_PGS	*Hafnia alvei* strain FDAARGOS_158, complete genome
1FH3	*Hafnia sp*. 3-12(2010) 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
2FH1_BHI	*Hafnia sp*. 3-12(2010) 16S ribosomal RNA gene, partial sequence
2FM3_BHI	*Hafnia sp.* 3-12(2010) 16S ribosomal RNA gene, partial sequence
2FM3_PGS	*Hafnia sp*. 3-12(2010) 16S ribosomal RNA gene, partial sequence
Man._F._hind._1-2	*Hafnia sp*. 3-12(2010) 16S ribosomal RNA gene, partial sequence
Mannitol_FH1	*Hafnia sp.* 3-12(2010) 16S ribosomal RNA gene, partial sequence
1FM1	*Hafnia sp*. B315 16S ribosomal RNA gene, partial sequence
2FM1_BHI	*Hafnia sp*. B315 16S ribosomal RNA gene, partial sequence
Faek2_PGS	*Hafnia sp*. B315 16S ribosomal RNA gene, partial sequence
FF9_H20_BHI	*Hafnia sp*. B315 16S ribosomal RNA gene, partial sequence
3MM1	*Lactococcus garvieae* ATCC 49156 strain ATCC 49156 16S ribosomal RNA
2FH3_PGS	*Lactococcus garvieae* partial 16S rRNA gene, strain qz-367
2MH3-3_BHI	*Lactococcus garvieae* strain H25 16S ribosomal RNA gene, partial sequence
2MH3-1_BHI	*Lactococcus garvieae* strain JB275804/2 16S ribosomal RNA gene, partial sequence
2FM4-2_BHI	*Lactococcus garvieae* strain JB282647 2 16S ribosomal RNA gene, partial sequence
2MF2-1_BHI	*Lactococcus garvieae* strain JB282647 2 16S ribosomal RNA gene, partial sequence
2MM3_BHI	*Lactococcus lactis* strain FT697 16S ribosomal RNA gene, partial sequence
2FH3	*Lactococcus lactis* strain Guimu 24 16S ribosomal RNA gene, partial sequence
3	*Leifsonia lichenia* strain 2Sb 16S ribosomal RNA gene
51	*Microbacterium oxydans* strain Abk8 16S ribosomal RNA gene, partial sequence
30	*Microbacterium sp*. y2 16S ribosomal RNA gene, partial sequence
31	*Microbacterium sp*. y2 16S ribosomal RNA gene, partial sequence
WH_einzel10^7	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
Wild_Anal_1	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Anal_2	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Anal_3	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Anal_4	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Hind_2	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Hind_4	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Hind_einzel	*Morganella morganii* strain wf-1 16S ribosomal RNA gene, partial sequence
Wild_Anal_6	*Morganella morganii* strain ZW45-1 16S ribosomal RNA gene, partial sequence
3MM2	*Morganella morganii* subsp. *morganii* KT 16S ribosomal RNA
Wild_Hind_3	*Morganella morganii* subsp. *morganii* KT, complete genome
Wild_Hind_5	*Morganella morganii* subsp. *morganii* KT, complete genome
2AB_MH1	*Myroides odoratus* strain 154 (C4Plas©) 16S ribosomal RNA gene, partial sequence
2AB_MH2	*Myroides odoratus* strain 154 (C4Plas©) 16S ribosomal RNA gene, partial sequence
2AB_MH4	*Myroides odoratus* strain 154 (C4Plas©) 16S ribosomal RNA gene, partial sequence
55	*Nocardioides oleivorans* gene for 16S rRNA, partial sequence
10	*Ochrobactrum sp*. KAR47 16S ribosomal RNA gene, complete sequence
35	*Ochrobactrum sp*. KAR47 16S ribosomal RNA gene, complete sequence
YGC-2	*Penicillium lagena* gene for 18S rRNA, partial sequence
Wild_Mid_12	*Proteus mirabilis* strain AER311-8 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
Wild_Mid_6	*Proteus mirabilis* strain AER311-8 16S ribosomal RNA gene, partial sequence
Wild_Mid_18	*Proteus mirabilis* strain QHD 16S ribosomal RNA gene, partial sequence
Wild_Mid_7	*Proteus mirabilis* strain QHD 16S ribosomal RNA gene, partial sequence
Wild_Hind_1	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
Wild_Mid_10	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
Wild_Mid_11	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
Wild_Mid_13	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
Wild_Mid_15	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
Wild_Mid_20	*Proteus mirabilis* strain R2 16S ribosomal RNA gene, partial sequence
101	*Proteus sp*. 632B2_12AEMB 16S ribosomal RNA gene, partial sequence
100	*Proteus sp.* BUR10 16S ribosomal RNA gene, partial sequence
102	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
103	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
104	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
105	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
Wild_Mid_16	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
Wild_Mid_21	*Proteus sp*. BUR10 16S ribosomal RNA gene, partial sequence
3MA1	*Providencia rettgeri* strain DSM 4542
3MH3	*Providencia rettgeri* strain DSM 4542
2FH2	*Providencia sp*. BAB-5308 16S ribosomal RNA gene, partial sequence
1FH2	*Providencia sp*. BIHB 1402 16S ribosomal RNA gene, partial sequence
1FM2	*Providencia sp*. BIHB 1402 16S ribosomal RNA gene, partial sequence
1MH1	*Providencia sp.* BIHB 1402 16S ribosomal RNA gene, partial sequence
3MA2	*Pseudochrobactrum kiredjianiae* strain CCUG 49584
2AB_FF1	*Pseudochrobactrum saccharolyticum* strain ALK626 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
2AB_FF2	*Pseudochrobactrum saccharolyticum* strain ALK626 16S ribosomal RNA gene, partial sequence
2AB_FF7	*Pseudochrobactrum saccharolyticum* strain ALK626 16S ribosomal RNA gene, partial sequence
93	*Pseudochrobactrum saccharolyticum* strain W14 16S ribosomal RNA gene, partial sequence
2AB_FF6	*Pseudochrobactrum sp*. H-VRE-110b 16S ribosomal RNA gene, partial sequence
80	*Pseudomonas brenneri* strain BD09-00307 16S ribosomal RNA gene, partial sequence
85	*Pseudomonas brenneri* strain BD09-00307 16S ribosomal RNA gene, partial sequence
81	*Pseudomonas extremorientalis* strain NSPtBx02 16S ribosomal RNA gene, partial sequence
60	*Pseudomonas fluorescens strain n10 16S ribosomal RNA gene, partial sequence*
65	*Pseudomonas fluorescens* strain n10 16S ribosomal RNA gene, partial sequence
26	*Pseudomonas fluorescens* strain PCL1751, complete genome
62	*Pseudomonas fragi* strain F26 16S ribosomal RNA gene, partial sequence
27	*Pseudomonas sp.* 27Kp1 16S ribosomal RNA gene, partial sequence
90	*Pseudomonas sp*. 27Kp1 16S ribosomal RNA gene, partial sequence
88	*Pseudomonas sp*. 35_2 16S ribosomal RNA gene, partial sequence
57	*Pseudomonas sp*. BTN10 16S ribosomal RNA gene, partial sequence
84	*Pseudomonas sp*. FBF110 partial 16S rRNA gene, isolate FBF110
86	*Pseudomonas sp*. LC182 16S ribosomal RNA gene, partial sequence
66	*Pseudomonas sp*. n5(2012) 16S ribosomal RNA gene, partial sequence
58	*Pseudomonas sp*. PP103 16S ribosomal RNA gene, partial sequence
20	*Pseudomonas sp.* P-W-3 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
44	*Rhodococcus qingshengii* strain PVL12 16S ribosomal RNA gene, partial sequence
Feak1_PGS	*Serratia ficaria* strain YJ1 16S ribosomal RNA gene, complete sequence
Mannitol_MF4	*Serratia liquefaciens* strain 24K11 16S ribosomal RNA gene, partial sequence
Man._M._for._2-2	*Serratia liquefaciens* strain FDAARGOS_125, complete genome
97	*Serratia liquefaciens* strain KAR19 16S ribosomal RNA gene, complete sequence
2MF1_PGS	*Serratia marcescens* strain 16DR 16S ribosomal RNA gene, partial seq.
2MH1_PGS	*Serratia marcescens* strain IARI-CRK 16 16S ribosomal RNA gene, partial sequence
2MH3-2_BHI	*Serratia marcescens* strain N1.14 16S ribosomal RNA gene, partial sequence
2FH4_BHI	*Serratia marcescens* strain S20 16S ribosomal RNA gene, partial sequence
Mannitol_MF5	*Serratia marcescens subsp*. *sakuensis* strain RN25 16S ribosomal RNA gene, partial sequence
2MH1_BHI	*Serratia marcescens subsp*. *sakuensis* strain RY21 16S ribosomal RNA gene, partial sequence
2MH2-1_BHI	*Serratia marcescens subsp*. *sakuensis* strain RY21 16S ribosomal RNA gene, partial sequence
FF6_H20_BHI	*Serratia plymuthica* strain KAR17 16S ribosomal RNA gene, complete sequence
56	*Serratia plymuthica* strain UBCR_12 16S ribosomal RNA gene, partial sequence
87	*Serratia proteamaculans* partial 16S rRNA gene, strain TRS1-WB
15	*Serratia proteamaculans* strain 1Cg-44 16S ribosomal RNA gene, partial sequence
2	*Serratia proteamaculans* strain B34 16S ribosomal RNA gene, partial sequence
16	*Serratia proteamaculans* strain B34 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
23	*Serratia proteamaculans* strain B34 16S ribosomal RNA gene, partial sequence
52	Serratia proteamaculans strain G32 16S ribosomal RNA gene, partial sequence
Feak3_BHI	*Serratia sp*. #YN-5 gene for 16S ribosomal RNA, partial sequence
18	*Serratia sp*. 73 16S ribosomal RNA gene, partial sequence
28	*Serratia sp*. 73 16S ribosomal RNA gene, partial sequence
67	*Serratia sp*. 73 16S ribosomal RNA gene, partial sequence
61	*Serratia sp*. BACM14 16S ribosomal RNA gene, partial sequence
1	*Serratia sp*. JW65.6a partial 16S rRNA gene, strain JW65.6a
17	*Serratia sp*. KB17 16S ribosomal RNA gene, partial sequence
78	*Serratia sp*. KB17 16S ribosomal RNA gene, partial sequence
83	*Serratia sp*. KB17 16S ribosomal RNA gene, partial sequence
91	*Serratia sp*. KB17 16S ribosomal RNA gene, partial sequence
9	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
33	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
43	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
47	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
59	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
73	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
74	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
75	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
6	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
Mannitol_MF9	*Serratia sp*. L0305 16S ribosomal RNA gene, partial sequence
Faek1_PGS	*Serratia sp*. PSTB2 16S ribosomal RNA gene, partial sequence
Mannitol_FM2	*Serratia sp.* Q3 16S ribosomal RNA gene
63	*Serratia sp.* TWJ22 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
Faek3_PGS	*Serratia sp*. UA-JF0212 16S ribosomal RNA gene, partial sequence
Feak1-2	*Serratia sp*. UA-JF0212 16S ribosomal RNA gene, partial sequence
71	*Stenotrophomonas humi* strain R-32729 16S ribosomal RNA gene
AB4	*Stenotrophomonas maltophilia* 16S ribosomal RNA gene, partial sequence
2AB_MF1	*Stenotrophomonas maltophilia* strain 261ZG10 16S ribosomal RNA gene, partial sequence
2AB_MM1	*Stenotrophomonas maltophilia* strain 261ZG10 16S ribosomal RNA gene, partial sequence
2AB_MF3	*Stenotrophomonas maltophilia* strain 262XG3 16S ribosomal RNA gene, partial sequence
53	*Stenotrophomonas maltophilia* strain 262XG5 16S ribosomal RNA gene, partial sequence
21	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
92	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
94	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
96	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
99	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
2AB_FH2	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
2AB_FH3	*Stenotrophomonas maltophilia* strain 2Kp7a 16S ribosomal RNA gene, partial sequence
41	*Stenotrophomonas maltophilia* strain 4K1A 16S ribosomal RNA gene, partial sequence
49	*Stenotrophomonas maltophilia* strain 4K1A 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
AB5	*Stenotrophomonas maltophilia* strain D1 16S ribosomal RNA gene, partial sequence
69	*Stenotrophomonas maltophilia* strain LZC3 16S ribosomal RNA gene, partial sequence
2AB_FF3	*Stenotrophomonas maltophilia* strain LZC3 16S ribosomal RNA gene, partial sequence
2AB_FF4	*Stenotrophomonas maltophilia* strain LZC3 16S ribosomal RNA gene, partial sequence
2AB_FF5	*Stenotrophomonas maltophilia* strain LZC3 16S ribosomal RNA gene, partial sequence
32	*Stenotrophomonas maltophilia* strain QH31 16S ribosomal RNA gene, partial sequence
3MA3	*Stenotrophomonas pavanii* strain LMG 25348
46	*Stenotrophomonas sp*. 4NR6 16S ribosomal RNA gene, partial sequence
2AB_FH1	*Stenotrophomonas sp*. 4NR6 16S ribosomal RNA gene, partial sequence
72	*Stenotrophomonas sp*. CanL-50 16S ribosomal RNA gene, partial sequence
64	*Stenotrophomonas sp*. DA1 16S ribosomal RNA gene, partial sequence
98	*Stenotrophomonas sp. SB341 16S ribosomal RNA gene, partial sequence*
3FA1	*Streptomyces herbaricolor* strain NBRC 3932
3MH2	*Vagococcus fessus* strain m2661/98/1
3MA4	*Vagococcus fessus* strain m2661/98/1 16S ribosomal RNA gene
3MM3	*Vagococcus fessus* strain m2661/98/1 16S ribosomal RNA gene
29	*Variovorax boronicumulans* partial 16S rRNA gene, isolate 0911ARD9I2
34	*Variovorax sp*. MG3 16S ribosomal RNA gene, partial sequence
3FM1	*Wohlfahrtiimonas larvae* strain KBL006 16S ribosomal RNA
AB3	*Yarrowia divulgata* isolate F6-17 18S ribosomal RNA gene
YGC-6	*Yarrowia lipolytica* partial 23S rRNA gene, strain K
YGC-1	*Yarrowia lipolytica* strain ATCC 9773 18S ribosomal RNA gene

Strain ID	Best BLASTn: closest genetic relative available
YGC-19	*Yarrowia lipolytica* strain ATCC 9773 18S ribosomal RNA gene
YGC-4	*Yarrowia lipolytica* strain ATCC 9773 18S ribosomal RNA gene
YGC-7	*Yarrowia lipolytica* strain ATCC 9773 18S ribosomal RNA gene
YGC-11	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-13	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-14	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-16	*Yarrowia sp.* D30L 18S ribosomal RNA gene, partial sequence
YGC-26	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-27	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-5	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-8	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
YGC-9	*Yarrowia sp*. D30L 18S ribosomal RNA gene, partial sequence
4	Uncultured bacterium clone 10-472 16S ribosomal RNA gene, partial sequence
2MF5_BHI	Uncultured bacterium clone 8817-D40-PY-O-1B 16S ribosomal RNA gene, partial sequence
77	Uncultured bacterium clone C6 16S ribosomal RNA gene, partial sequence
Man._F._hind._4	Uncultured bacterium clone C6 16S ribosomal RNA gene, partial sequence
12	Uncultured bacterium clone KNB33 16S ribosomal RNA gene, partial sequence
50	Uncultured bacterium clone KNB33 16S ribosomal RNA gene, partial sequence
2AB_MF2	Uncultured bacterium clone LF4FR5D05 16S ribosomal RNA gene, partial sequence
2MH4_BHI	Uncultured bacterium clone MgKI1c003b02 16S ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
48	Uncultured bacterium clone RL185_aaj72c11 16S ribosomal RNA gene, partial sequence
a-12	Uncultured bacterium clone rRNA198 16S ribosomal RNA gene, partial sequence
2AB_FH4	Uncultured bacterium clone SINI672 16S ribosomal RNA gene, partial sequence
2MF3_BHI	Uncultured bacterium clone SINI836 16S ribosomal RNA gene, partial sequence
2FM5_PGS	Uncultured bacterium partial 16S rRNA gene, amplicon K
79	Uncultured gamma proteobacterium clone FTLpost17 16S ribosomal RNA gene, partial sequence
2MH3-1_PGS	Uncultured *Lactococcus sp*. clone S1_G08 16S ribosomal RNA gene, partial sequence
2MH3-3_PGS	Uncultured *Lactococcus sp*. clone S5_A12 16S ribosomal RNA gene, partial sequence
2MM2_BHI	Uncultured *Lactococcus sp*. gene for 16S rRNA, partial sequence, clone: CLZX63
54	Uncultured *Microbacterium* sp. clone KNB18 16S ribosomal RNA gene, partial sequence
2MH3-2_PGS	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000142 small subunit ribosomal RNA gene, partial sequence
Mannitol_MF1	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000257 small subunit ribosomal RNA gene, partial sequence
1FF1	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence
2FH4-2_BHI	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence
2FH5	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence
2FM2_PGS	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence

Strain ID	Best BLASTn: closest genetic relative available
FF8_H20_BHI	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence
FF9_H2O	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000327 small subunit ribosomal RNA gene, partial sequence
MH2	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000450 small subunit ribosomal RNA gene, partial sequence
1MF1	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
1MF2	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
1MM2	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
2FM4-1_BHI	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
2MF2-2_BHI	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
Mannitol_MF6	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
Mannitol_MF7	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
MH3	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
MM1	Uncultured organism clone ELU0075-T355-S-NIPCRAMgANa_000498 small subunit ribosomal RNA gene, partial sequence
Wild_Mid_14	Uncultured organism clone ELU0159-T329-S-NIPCRAMgANa_000317 small subunit ribosomal RNA gene, partial sequence
2MH3-3_PGS	unknown
3MH1	96%: *Wohlfahrtiimonas larvae* strain KBL006 16S ribosomal RNA
3MA6	97,6% *Vagococcus fessus* strain m2661/98/1 16S ribosomal RNA gene
3MA7	98% *Vagococcus fessus* strain m2661/98/1 16S ribosomal RNA gene

Table S2: antimicrobial effects of all screened extracts

Strain	growth inhibition [%]											
	Ecol ATCC25922		Ecol dTolC		Paer ATCC27853		Saur ATCC25923		C. albicans FH2173		Msme ATCC 709	
	10	10	10	10	10	10	10	10	10	10	10	10
10% BHI MeOH	12	15	6	7	-15	-20	-66	-95	-32	-14	33	33
10% BHI EtOAc	31	-5	34	39	6	-1	1	2	9	-20	23	2
TBS EtOH	29	9	-1	18	-2	7	-16	-60	0	0	0	0
TBS MeOH	23	-5	-23	11	-10	-4	-36	-85	0	0	0	0
8 d1 MeOH	-6	6	-1	9	-127	-60	-107	-124	-152	-172	53	62
8 d1 EtOAc	31	46	45	41	-3	-10	-19	-17	-53	-70	15	5
8d6 MeOH	23	-5	24	28	-22	-27	-105	-150	-147	-166	65	33
8d6 EtOAc	16	-9	-13	-5	-4	3	7	3	5	-12	40	23
8d9 MeOH	-31	-24	-23	-5	-36	-16	-49	-86	-157	-125	34	39
8d9 EtOAc	3	0	14	13	3	-12	-2	-2	-12	-138	-7	24
13d1 MeOH	20	34	58	67	-9	-50	-91	-115	-65	-77	77	70
13d1 EtOAc	1	-7	24	43	-11	-63	4	-6	-15	-101	-16	2
13d6 MeOH	24	27	42	34	10	-63	-48	-53	11	66	81	81
13d6 EtOAc	15	2	12	1	-39	-15	-5	2	-28	43	43	35
13d9 MeOH	18	20	24	15	-3	-8	-46	-54	-21	-72	80	79
13d9 EtOAc	7	-5	-6	-13	14	-18	2	-4	-39	-58	1	37
45d1 MeOH	24	-24	7	26	-7	-23	-113	-122	-120	-182	63	54
45d1 EtOAc	6	-5	32	-2	-6	0	0	-11	-80	-136	2	0
45d6 MeOH	-5	11	23	20	-13	-18	-57	-72	-104	-111	73	72
45d6 EtOAc	30	29	12	15	-4	5	4	-2	39	-73	25	-9
45d9 MeOH	-2	2	14	12	-17	-19	-66	-79	-58	2	62	59
45d9 EtOAc	30	43	40	35	-4	-18	-6	-21	19	-18	4	-8
70d1 MeOH	-3	3	-9	-5	-35	-28	-76	-90	-91	-132	44	27
70d1 EtOAc	20	-11	4	-8	3	8	-4	-15	38	-15	-18	-15
70d6 MeOH	-12	11	16	13	-9	-11	-35	-30	-105	-106	24	32
70d6 EtOAc	8	10	7	-10	4	-23	0	-7	-11	-18	16	-19
70d9 MeOH	-14	6	6	-7	-12	-72	-41	-51	-71	-110	75	71
70d9 EtOAc	-16	-7	-12	-5	1	3	4	-24	-30	-98	-13	-18
AB1d1 MeOH	7	22	74	56	-104	-144	-70	-76	-25	-93	9	-19
AB1d1 EtOAc	6	20	13	-2	-11	-3	-30	-60	-10	-105	-17	-25
AB1d6 MeOH	5	38	51	51	-65	-44	-60	-90	-35	-23	2	1
AB1d6 EtOAc	4	19	16	21	15	-2	-50	-62	-127	-50	-7	-4
AB1d9 MeOH	0	19	23	30	-37	-41	-61	-87	-4	-10	-3	-20
AB1d9 EtOAc	-3	13	9	-8	-15	-10	-46	-62	-112	-71	-10	-29
AB2d1 MeOH	-6	7	11	6	-21	-21	-104	-88	-117	-45	26	20
AB2d1 EtOAc	-14	6	9	3	-5	-8	-1	-35	-9	-48	18	8
AB2d6 MeOH	13	4	26	13	-20	-48	-105	-125	20	20	38	35
AB2d6 EtOAc	-15	-1	-1	5	-10	-8	-7	-12	7	-2	22	16
AB2d9 MeOH	-34	-9	10	19	-20	-20	-72	-106	20	-58	31	26
AB2d9 EtOAc	-19	2	-1	5	-6	-4	-1	-9	3	22	17	23
1FF2d1 MeOH	4	21	33	32	-9	-12	-87	-114	-73	-140	-56	-67
1FF2d1 EtOAc	25	21	33	25	-10	-9	-11	-12	-68	-76	-20	3

1FF2d6 MeOH	-2	12	6	10	-34	-14	-75	-87	8	-163	63	62
1FF2d6 EtOAc	-7	3	19	-9	5	-7	-3	-14	-31	-54	-29	-32
1FF2d9 MeOH	-6	7	29	13	-18	-24	-63	-82	-90	-54	74	77
1FF2d9 EtOAc	-20	-6	-4	-2	14	-5	-6	-9	17	-30	-28	25
1MM3d1 MeOH	5	15	7	-3	-29	-37	-6	-49	-52	-39	-2	-4
1MM3d1 EtOAc	17	-4	36	-2	-3	-9	0	-27	-62	42	-48	-51
1MM3d6 MeOH	2	20	16	17	-2	-21	-63	-102	11	-103	28	34
1MM3d6 EtOAc	18	-7	29	-8	-8	-9	-24	-16	-18	-40	-43	-47
1MM3d9 MeOH	15	28	24	18	-20	-16	-45	-75	-38	-47	27	24
1MM3d9 EtOAc	21	31	36	32	-7	6	-11	-16	-36	-19	-31	-21
10d1 MeOH	11	12	15	15	-137	-34	-117	-98	-90	-91	73	68
10d1 EtOAc	29	34	35	29	-3	-9	-85	-60	-102	-131	-24	12
10d6 MeOH	15	27	15	28	-89	-19	-108	-110	-89	-113	83	80
10d6 EtOAc	23	-3	26	-5	-37	10	3	9	10	-27	-25	-7
10d9 MeOH	16	15	15	17	-56	-3	-114	-109	-41	-98	82	84
10d9 EtOAc	33	12	40	38	12	1	-8	10	-33	-51	-21	-4
30d1 MeOH	2	4	7	6	-17	-99	-125	-110	-87	-107	45	70
30d1 EtOAc	38	37	38	30	-6	-6	-23	-10	-2	-67	15	13
30d6 MeOH	31	35	32	32	-8	-4	-85	-79	-4	-76	82	85
30d6 EtOAc	0	2	3	-2	6	5	-2	10	14	-20	-1469	-19
30d9 MeOH	19	25	26	21	-72	-11	-112	-75	60	38	82	87
30d9 EtOAc	-7	11	24	7	12	-3	5	10	9	-50	-17	-1
36d1 MeOH	18	27	17	23	-9	-33	-140	-115	-69	-52	57	57
36d1 EtOAc	45	42	35	33	3	-14	-4	6	10	-84	-16	-19
36d6 MeOH	11	0	8	6	-24	-35	-120	-107	-98	-92	46	57
36d6 EtOAc	-8	-12	19	-1	1	-1	-2	8	0	-82	0	0
36d9 MeOH	15	17	18	18	-13	-32	-85	-85	-40	-70	-72	-27
36d9 EtOAc	8	38	35	31	2	0	-13	4	-62	67	15	24
51d1 MeOH	7	17	22	9	-42	-158	-95	-65	-115	-90	71	77
51d1 EtOAc	1	42	32	27	-18	-9	-12	-7	41	44	-39	-2
51d6 MeOH	20	19	21	21	-36	-131	-122	-101	-124	-54	85	88
51d6 EtOAc	25	34	32	34	-6	-10	-9	2	-35	-24	-15	-10
51d9 MeOH	15	18	17	17	-165	-1	-110	-68	-98	-88	87	84
51d9 EtOAc	-8	38	35	28	-11	-19	-15	2	-53	33	-35	-18
1FH3d1 MeOH	26	26	48	39	-24	-36	-92	-100	-55	-41	25	19
1FH3d1 EtOAc	21	40	26	32	-4	-11	-13	-6	-89	5	-8	99
1FH3d6 MeOH	-2	-11	46	36	-25	-40	-93	-50	-35	-47	84	81
1FH3d6 EtOAc	21	29	28	30	-4	-8	-10	1	14	-12	25	16
1FH3d9 MeOH	23	32	94	83	-40	-46	-81	-70	-9	8	87	89
1FH3d9 EtOAc	-4	3	11	2	-8	-9	-16	4	-17	22	-46	24
2FM1d1 MeOH	31	27	37	41	-35	-46	-106	-96	5	-31	54	58
2FM1d1 EtOAc	17	28	24	22	-12	-19	-14	-7	-120	7	-26	-2
2FM1d6 MeOH	16	18	80	57	-42	-73	-90	-83	-43	-11	96	94
2FM1d6 EtOAc	14	-1	1	-3	-10	-16	-9	6	7	-67	-37	-17
2FM1d9 MeOH	40	7	90	88	-45	-47	-77	-72	65	43	95	94
2FM1d9 EtOAc	-2	-8	10	3	-5	-12	-12	3	14	10	22	-1
2MM1d1 MeOH	16	16	14	14	-36	-38	-103	-94	-20	-45	31	59

2MM1d1 EtOAc	17	1	26	29	-8	-4	-23	-16	-16	-37	13	22
2MM1d6 MeOH	4	11	4	3	-22	-30	-53	-86	-77	-34	65	58
2MM1d6 EtOAc	41	39	22	35	-12	-15	-12	-6	-46	-31	-12	-5
2MM1d9 MeOH	41	28	39	19	-41	-47	-70	-46	60	-108	50	54
2MM1d9 EtOAc	21	17	27	16	-12	-19	-19	-1	-14	-39	-31	-16
2MM2-2d1 MeOH	12	14	12	8	-31	-36	-109	-100	71	41	25	45
2MM2-2d1 EtOAc	5	6	4	-1	-10	-9	-1	4	15	-15	-41	-4
2MM2-2d6 MeOH	11	10	14	7	-26	-14	-55	-58	59	76	44	48
2MM2-2d6 EtOAc	-10	-10	8	13	-1	-9	-1	-1	40	42	-18	6
2MM2-2d9 MeOH	11	10	13	8	-35	-30	-80	-70	14	-12	31	50
2MM2-2d9 EtOAc	33	30	32	37	-11	-27	-8	-9	11	-1	14	23
2MH3-2d1 MeOH	19	12	10	2	-22	-30	30	-21	-50	15	77	76
2MH3-2d1 EtOAc	26	29	22	28	5	-6	-26	-15	-67	17	-19	3
2MH3-2d6 MeOH	9	9	10	-12	-31	-40	-48	65	-7	-43	43	54
2MH3-2d6 EtOAc	26	31	20	24	-7	-41	-31	-17	-13	-52	-27	-10
2MH3-2d9 MeOH	8	14	8	7	-30	-49	99	72	-8	-34	102	101
2MH3-2d9 EtOAc	24	24	17	18	-25	-8	-30	-25	-115	-29	-34	-18
2FH3d1 MeOH	3	19	2	-4	-36	-42	-110	-84	25	1	53	58
2FH3d1 EtOAc	2	36	17	4	13	-6	-2	13	-95	26	-1	-10
2FH3d6 MeOH	12	14	40	30	100	99	-20	35	-64	-104	58	52
2FH3d6 EtOAc	5	27	19	25	4	2	-16	-4	18	-36	14	14
2FH3d9 MeOH	-13	-27	25	23	-16	-50	-38	-42	-2	-90	58	48
2FH3d9 EtOAc	-6	-12	2	7	-8	7	-2	-7	43	29	-17	-5
3 d1 EtOAc	36	33	44	42	49	36	-20	-31	-53	-77	18	-28
3 d1 MeOH	6	7	6	8	-86	-63	-25	-92	-38	-43	74	49
3 d6 EtOAc	42	44	24	39	-12	-9	-25	-57	-37	-34	-29	-24
3 d6 MeOH	2	4	18	14	-92	-123	-42	-97	-39	-56	73	66
3 d9 EtOAc	-2	-16	-13	-22	-22	-30	17	-21	-7	-7	4	7
3 d9 MeOH	0	-2	2	-20	-45	-39	-47	-100	-53	-83	71	76
15 d1 EtOAc	24	28	12	6	14	7	-3	-18	-46	-43	-17	9
15 d1 MeOH	20	24	39	26	-105	-16	-21	-92	-77	-83	81	77
15 d6 EtOAc	26	24	27	28	-13	-6	19	-12	-55	-34	-45	-10
15 d6 MeOH	15	16	17	17	-149	-100	-44	-78	-42	-64	86	82
15 d9 EtOAc	-13	4	21	7	-8	2	11	-7	-17	-27	-11	-30
15 d9 MeOH	15	14	24	18	-40	-26	-37	-97	-33	-66	94	90

16 d1 EtOAc	34	34	32	35	-16	-19	19	-16	-44	-47	-75	-71
16 d1 MeOH	18	15	25	18	-12	0	-15	-42	-8	-32	84	82
16 d6 EtOAc	2	23	-2	13	-5	1	14	5	-17	-22	-13	-39
16 d6 MeOH	16	17	8	1	-9	-18	-45	-92	24	17	41	77
16 d9 EtOAc	3	-16	0	18	-5	-5	36	11	-29	-48	-16	-28
16 d9 MeOH	16	21	19	11	-20	-27	-36	-67	-13	6	22	44
27 d1 EtOAc	29	24	19	23	-7	-14	0	-18	-11	-41	-16	-22
27 d1 MeOH	9	7	22	5	8	-6	-1	-69	14	-6	82	60
27 d6 EtOAc	-1	2	5	-16	4	7	26	19	2	-21	-14	-48
27 d6 MeOH	7	1	15	21	2	-4	-33	-96	-7	-19	83	81
27 d9 EtOAc	-10	-3	-11	-4	-3	3	29	4	-10	-13	-7	-38
27 d9 MeOH	20	21	21	17	-4	-6	-21	-101	1	-11	80	76
44 d1 EtOAc	-3	16	16	-7	8	7	46	22	1	-38	-11	-16
44 d1 MeOH	-22	-21	-41	-25	-24	-24	-15	-69	-28	-57	73	80
44 d6 EtOAc	17	34	-10	-6	-5	-5	19	3	-18	-15	25	10
44 d6 MeOH	8	16	18	9	15	8	-32	-94	-52	-75	93	92
44 d9 EtOAc	-5	29	16	28	0	6	35	8	26	-16	-29	17
44 d9 MeOH	11	15	17	9	3	-6	-31	-89	-13	-39	81	81
52 d1 EtOAc	14	35	34	34	-20	-12	19	-13	-14	-30	-17	-47
52 d1 MeOH	21	21	18	21	-24	-4	11	-13	0	-23	34	55
52 d6 EtOAc	8	16	22	21	1	-5	34	8	-37	-47	-51	-40
52 d6 MeOH	22	21	9	-2	-4	-12	-29	-66	5	6	72	77
52 d9 EtOAc	-12	-13	4	2	-3	4	37	3	-32	-51	16	-8
52 d9 MeOH	19	23	23	14	-11	-7	-4	-61	31	13	68	73
55 d1 EtOAc	25	39	20	19	1	6	36	6	-6	-50	10	4
55 d1 MeOH	5	15	11	-1	-20	-23	-30	-87	7	63	70	73
55 d6 EtOAc	-4	28	19	13	-3	-2	29	13	-4	-6	0	-27
55 d6 MeOH	4	3	8	-1	-14	-2	-16	-75	-19	-54	79	76
55 d9 EtOAc	31	35	34	21	-20	-14	7	-31	-50	-62	-27	-45
55 d9 MeOH	9	19	30	28	-26	-26	-33	-112	-6	-4	67	67
60 d1 EtOAc	32	30	45	29	-15	-9	20	-40	-46	-65	-45	-66
60 d1 MeOH	23	22	31	25	-11	-8	-35	-95	-24	-38	56	67

60 d6 EtOAc	30	36	38	27	-19	-9	2	-45	-67	-85	-21	-27
60 d6 MeOH	19	26	28	19	-19	-15	-24	-73	24	25	98	97
60 d9 EtOAc	38	30	34	26	-16	-16	26	-5	-32	-49	34	14
60 d9 MeOH	11	26	23	12	-18	-19	-27	-85	-34	-72	97	96
62 d1 EtOAc	26	45	23	3	-22	-20	1	-38	-6	-50	-39	-19
62 d1 MeOH	9	18	10	17	-13	-29	-38	-107	-36	-59	75	72
62 d6 EtOAc	13	16	19	25	0	-3	-9	-42	-9	-43	25	2
62 d6 MeOH	21	20	24	22	-26	-27	-39	-81	-25	-9	96	97
62 d9 EtOAc	13	26	28	20	-11	-6	24	-2	-19	-38	20	-20
62 d9 MeOH	24	21	24	20	-22	-5	-14	-61	20	22	97	97
66 d1 EtOAc	35	38	31	31	-14	-18	32	5	-22	-57	6	10
66 d1 MeOH	11	17	30	24	-10	-27	-31	-93	1	-76	59	62
60 d6 EtOAc	33	41	37	32	-20	-15	26	-5	-17	-36	29	2
66 d6 MeOH	13	35	21	12	-1	-40	-30	-95	-38	-87	98	97
66 d9 EtOAc	10	21	16	4	-2	-1	36	20	12	-22	7	12
66 d9 MeOH	16	16	16	9	-9	-13	-28	-73	-21	-46	96	96
80 d1 EtOAc	31	30	42	8	-22	-17	-12	-47	-49	-80	-68	-13
80 d1 MeOH	14	14	18	15	-5	-5	-36	-83	-18	-56	51	77
80 d6 EtOAc	32	33	43	35	-20	-20	-4	-44	-53	-82	-40	-44
80 d6 MeOH	11	13	19	11	-16	-18	-40	-103	-41	-91	99	97
80 d9 EtOAc	32	39	20	3	-13	-18	22	-3	-15	-59	4	22
80 d9 MeOH	8	12	22	14	4	-9	-31	-88	-4	-77	97	96
81 d1 EtOAc	20	39	35	19	-22	-12	-4	-39	-66	-71	-25	-34
81 d1 MeOH	10	10	13	6	-42	-38	-35	-94	-26	-67	71	65
81 d6 EtOAc	33	32	26	28	-19	-14	-8	-34	-42	-70	-24	-17
81 d6 MeOH	5	3	-16	8	-17	-17	-44	-118	4	-17	100	99
81 d9 EtOAc	35	36	34	33	-20	-13	-12	-36	-68	-62	5	-15
81 d9 MeOH	6	12	12	-1	4	-6	-43	-102	-15	-52	100	99
84 d1 EtOAc	33	33	33	22	-23	-17	-31	-53	-72	-76	3	-15
84 d1 MeOH	13	18	6	20	-20	-6	-44	-112	-14	-39	41	57
84 d6 EtOAc	33	37	27	15	-23	-13	5	-29	-54	-78	7	-6
84 d6 MeOH	1	8	11	-6	6	-12	-36	-96	-71	-70	96	97

84 d9 EtOAc	0	0	0	0	0	0	0	0	0	0	100	100
84 d9 MeOH	0	0	0	0	0	0	0	0	0	0	100	100
86 d1 EtOAc	36	32	17	36	-1	5	-66	-35	-20	-40	-62	-41
86 d1 MeOH	18	16	17	13	-8	-21	-120	-98	-47	-42	48	50
86 d6 EtOAc	43	20	36	33	-4	-6	-99	-57	-27	-22	-46	-53
86 d6 MeOH	5	4	98	93	-4	-2	-125	-82	-77	-73	97	99
86 d9 EtOAc	44	41	36	35	-2	-13	-43	-14	-20	-14	-40	-28
86 d9 MeOH	3	5	92	97	0	4	-123	-85	-77	-76	98	101
87 d1 EtOAc	40	36	7	22	-22	-18	-55	-25	-42	-42	-37	-42
87 d1 MeOH	21	21	30	22	7	-3	-133	-95	-41	-27	55	74
87 d6 EtOAc	5	34	-5	13	3	4	-9	5	-31	-12	-63	-69
87 d6 MeOH	19	18	22	21	-24	-19	-149	-89	20	7	71	71
87 d9 EtOAc	14	-1	11	10	5	1	-13	3	-32	-3	-90	-26
87 d9 MeOH	21	18	25	26	-19	-17	-129	-94	42	-5	67	63
101 d1 EtOAc	30	32	34	4	-3	2	-80	-31	-44	-32	-83	-41
101 d1 MeOH	15	13	18	14	-3	-16	-131	-95	-61	-54	77	71
101 d6 EtOAc	39	39	45	32	-3	-6	-61	-46	-28	-26	-41	-46
101 d6 MeOH	14	12	14	17	-9	-24	-124	-90	62	42	53	62
101 d9 EtOAc	20	-2	13	13	10	3	-57	7	-32	-6	-56	-26
101 d9 MeOH	2	-20	38	14	-23	-4	-147	-92	-59	-53	36	36
105 d1 EtOAc	32	23	41	37	-1	-4	-41	-14	-68	-79	-38	-90
105 d1 MeOH	-3	14	23	16	-18	1	-124	-91	-13	-42	66	67
105 d6 EtOAc	40	38	39	46	-9	-8	-76	-43	-24	-21	-56	-78
105 d6 MeOH	11	10	13	13	-15	-5	-130	-101	53	25	75	77
105 d9 EtOAc	11	10	11	13	-2	9	-12	-7	-34	-16	-7	-4
105 d9 MeOH	36	38	21	20	-19	-15	-124	-83	83	20	72	70
Wild Mid 10 d1 EtOAc	42	36	1	40	-13	-13	-73	-26	7	-9	-55	-51
Wild Mid 10 d1 MeOH	16	33	16	20	-15	-4	-124	-99	-44	-9	76	75
Wild Mid 10 d6 EtOAc	28	24	36	32	-14	-44	-97	-55	-84	-59	-85	-65
Wild Mid 10 d6 MeOH	16	13	15	16	-15	-14	-140	-108	13	-48	36	75
Wild Mid 10 d9 EtOAc	11	10	-1	30	7	3	-9	6	-17	7	-14	-29
Wild Mid 10 d9 MeOH	14	11	10	20	-12	-10	-114	-78	63	19	64	67

Wild Mid 12 d1 EtOAc	42	44	27	26	-10	-5	-60	-33	-17	-16	-20	-63
Wild Mid 12 d1 MeOH	10	9	18	14	-12	2	-125	-92	-34	-59	57	60
Wild Mid 12 d6 EtOAc	44	38	35	39	-10	-9	-84	-33	-26	-16	-39	-11
Wild Mid 12 d6 MeOH	16	2	11	12	-17	-8	-134	-100	29	23	57	74
Wild Mid 12 d9 EtOAc	37	32	25	33	-5	-9	-40	-8	-18	-15	-66	-54
Wild Mid 12 d9 MeOH	-5	10	12	19	-22	-12	-118	-66	82	34	76	74
Wild Mid 18 d1 EtOAc	36	32	35	43	-11	-10	-56	-12	-31	-23	-81	-89
Wild Mid 18 d1 MeOH	17	27	21	17	-13	-2	-113	-76	-11	-51	41	53
Wild Mid 18 d6 EtOAc	43	32	42	28	-16	-12	-56	-39	-41	3	-42	-82
Wild Mid 18 d6 MeOH	15	7	16	13	-52	-20	-132	-101	-69	-3	72	64
Wild Mid 18 d9 EtOAc	12	21	15	24	-7	-2	-22	1	-23	-3	-67	-24
Wild Mid 18 d9 MeOH	17	8	13	14	-29	-17	-126	-87	56	11	65	68
Wild Hind 5 d1 EtOAc	16	11	22	26	-3	-2	-46	-14	-35	-3	-29	-26
Wild Hind 5 d1 MeOH	14	13	28	22	-16	-14	-138	-97	72	44	49	51
Wild Hind 5 d6 EtOAc	29	6	22	21	-3	2	-19	4	-26	-22	-54	-53
Wild Hind 5 d6 MeOH	14	12	16	17	-14	-13	-126	-94	86	53	6	34
Wild Hind 5 d9 EtOAc	29	33	26	32	-12	-8	-23	-6	-33	-20	-62	-81
Wild Hind 5 d9 MeOH	26	27	42	24	-15	-14	-128	-99	-56	-54	64	66
Fäk1.1 d1 EtOAc	36	34	36	33	-12	-10	-65	-13	-52	-14	-78	-63
Fäk1.1 d1 MeOH	26	17	22	24	-28	-32	-115	-86	-15	-78	63	70
Fäk1.1 d6 EtOAc	22	24	19	11	-10	-15	-45	-10	-17	-5	-52	-31
Fäk1.1 d6 MeOH	26	28	28	25	-20	-17	-105	-74	-15	-53	12	13
Fäk1.1 d9 EtOAc	16	25	16	27	-8	-11	-23	-7	-51	-2	-71	-72
Fäk1.1 d9 MeOH	26	29	34	35	-27	-28	-106	-58	-54	-54	21	18
1FM2 d1 EtOAc	38	33	24	34	-9	-17	-65	-13	-107	-25	-80	-49
1FM2 d1 MeOH	13	14	23	17	-20	-21	-133	-91	7	-24	54	50
1FM2 d6 EtOAc	30	25	40	34	-14	-17	-83	-45	-78	-28	-56	-41
1FM2 d6 MeOH	17	14	13	10	-28	-22	-148	-112	-66	-61	-47	38
1FM2 d9 EtOAc	37	30	30	27	-18	-21	-48	-12	-49	-33	-5	-91
1FM2 d9 MeOH	14	12	14	15	-18	-10	-134	-101	37	-52	5	13
2MH1 d1 EtOAc	35	26	37	33	-16	-15	-35	-13	-37	-46	-100	-98
2MH1 d1 MeOH	12	17	22	17	-137	-23	82	79	-70	7	49	48

2MH1 d6 EtOAc	36	27	31	33	-9	-22	-40	-17	-33	-58	-88	-94
2MH1 d6 MeOH	16	18	19	20	-15	-15	16	63	69	-21	100	103
2MH1 d9 EtOAc	38	25	43	32	-7	-18	-38	-13	-42	-46	-92	-58
2MH1 d9 MeOH	8	9	14	12	-19	-25	-27	-14	76	29	101	104
2MH3-2 d1 EtOAc	22	20	27	18	-9	-7	71	59	-45	-52	41	41
2MH3-2 d1 MeOH	19	16	21	23	-12	-11	9	49	-98	-59	66	62
2MH3-2 d6 EtOAc	23	7	29	19	-4	32	-56	-35	-32	-59	-34	-77
2MH3-2 d6 MeOH	20	13	18	19	-28	-21	12	33	-55	-27	100	102
2MH3-2 d9 EtOAc	18	0	21	27	-3	13	-127	-66	2	-36	-16	-40
2MH3-2 d9 MeOH	15	13	20	19	-25	-34	16	57	-12	-38	101	104
2ABFF1 d1 EtOAc	43	25	8	10	-24	-26	-106	-90	-73	-89	-43	-37
2ABFF1 d1 MeOH	28	21	32	31	-34	-13	-155	-109	-63	-69	62	60
2ABFF1 d6 EtOAc	43	30	20	4	-27	-28	-84	-28	-71	-67	-62	-96
2ABFF1 d6 MeOH	25	19	8	27	-24	-12	-148	-111	-79	-92	54	39
2ABFF1 d9 EtOAc	0	0	0	0	0	0	0	0	0	0	100	100
2ABFF1 d9 MeOH	0	0	0	0	0	0	0	0	0	0	100	100
2ABFF6 d1 EtOAc	34	38	6	33	39	-9	-16	-45	-94	-42	1	-18
2ABFF6 d1 MeOH	23	22	32	34	-19	-15	-73	-62	-76	-66	49	49
2ABFF6 d6 EtOAc	29	34	38	31	34	15	-58	-57	-58	-79	-26	-46
2ABFF6 d6 MeOH	25	29	32	31	-18	-25	-84	-71	-92	-91	45	33
2ABFF6 d9 EtOAc	33	37	9	29	-2	-10	-19	-45	-60	-66	-34	-43
2ABFF6 d9 MeOH	24	26	39	36	-11	3	-84	-65	-77	-84	6	8
2ABMH1 d1 EtOAc	35	39	-2	1	-5	2	-32	-33	-78	-88	-27	5
2ABMH1 d1 MeOH	10	24	27	30	-2	-8	-12	-75	-81	-77	90	82
2ABMH1 d6 EtOAc	33	0	1	-5	-1	-1	3	13	4	-17	-20	-11
2ABMH1 d6 MeOH	-3	-1	34	7	-18	-18	-82	-62	-39	-66	75	66
2ABMH1 d9 EtOAc	41	-13	-15	-5	-7	4	15	14	-10	-17	-47	-41
2ABMH1 d9 MeOH	-11	-7	17	0	-44	-69	-80	-60	-47	-93	88	87
FF6-H2O d1 EtOAc	35	35	26	24	-2	4	0	1	-10	-32	62	59
FF6-H2O d1 MeOH	70	68	65	96	64	17	81	59	84	66	91	91
FF6-H2O d6 EtOAc	-10	12	-2	-3	-2	-6	-10	-1	-30	-55	-17	-23
FF6-H2O d6 MeOH	41	44	100	99	32	33	100	101	79	62	101	103

FF6-H2O d9 EtOAc	37	33	5	19	-5	-5	-4	3	-40	-47	-33	-39
FF6-H2O d9 MeOH	100	100	100	90	100	100	76	68	97	99	101	103
FF7-H2O d1 EtOAc	23	21	28	31	10	6	2	8	-83	-65	-73	-86
FF7-H2O d1 MeOH	10	15	11	11	-18	-2	-58	-59	-38	-91	79	66
FF7-H2O d6 EtOAc	31	25	43	31	-2	4	-27	-23	-48	-67	-48	-79
FF7-H2O d6 MeOH	6	20	16	14	-7	-3	-71	-60	46	57	76	48
FF7-H2O d9 EtOAc	27	31	5	29	-1	3	-47	-49	-49	-52	-58	-41
FF7-H2O d9 MeOH	12	18	18	14	-17	-26	-67	-61	44	65	57	21
Wild Anal 1 d1 EtOAc	36	37	29	21	-5	-1	-36	-32	-51	-78	-60	-57
Wild Anal 1 d1 MeOH	21	17	23	28	-3	-6	-71	-51	20	-25	43	48
Wild Anal 1 d6 EtOAc	21	30	36	37	0	-1	-45	-36	-49	-47	-29	-39
Wild Anal 1 d6 MeOH	5	13	7	5	-10	-2	-87	-66	31	-5	-21	-27
Wild Anal 1 d9 EtOAc	30	32	0	10	-7	-1	-9	-27	-13	-26	-20	-47
Wild Anal 1 d9 MeOH	10	12	9	6	-15	17	-64	-56	9	33	39	45
4 d1 EtOAc	41	35	-4	1	4	12	-14	-16	-106	-135	-40	18
4 d1 MeOH	5	5	-35	-17	-51	1	-121	-134	-141	-150	76	77
4 d6 EtOAc	40	38	9	-7	-1	-17	-10	-23	-78	-136	2	48
4 d6 MeOH	6	6	-56	-46	-46	-19	-109	-136	-109	-102	67	71
4 d9 EtOAc	40	40	13	6	3	13	-6	-12	-95	-100	-22	42
4 d9 MeOH	1	-1	-51	-41	-69	-47	-113	-145	-103	-106	54	63
20 d1 EtOAc	24	25	-8	-20	-21	8	-46	-69	-121	-32	-38	22
20 d1 MeOH	11	7	-28	-44	-38	3	40	62	69	76	78	87
20 d6 EtOAc	40	36	5	-7	-32	-2	-18	-13	-131	-137	-29	45
20 d6 MeOH	1	0	-50	-55	-6	3	-113	-135	-160	-1	99	100
20 d9 EtOAc	42	40	-12	-8	4	11	-13	-44	-112	-36	-14	12
20 d9 MeOH	12	9	-37	-49	-4	3	-114	-127	-131	12	97	99
26 d1 EtOAc	33	32	-7	-17	-24	-8	-26	-52	-230	-70	-42	8
26 d1 MeOH	12	4	-32	-42	-3	-41	-117	-145	-141	-118	64	79
26 d6 EtOAc	40	32	-1	-3	6	8	-10	-42	-172	-133	-20	30
26 d6 MeOH	7	3	-41	-49	4	9	-115	-135	-122	-87	99	100
26 d9 EtOAc	35	32	-11	-19	-8	10	-18	-38	-76	-52	-33	23
26 d9 MeOH	8	9	-43	-49	-26	3	-149	-140	-106	-123	99	98
29 d1 EtOAc	42	33	12	-15	1	-21	-2	-18	-152	-129	-56	27
29 d1 MeOH	0	2	-37	-43	91	82	-127	-121	-57	44	81	89
29 d6 EtOAc	37	19	1	-2	-5	5	-17	-23	-189	-175	101	102
29 d6 MeOH	-9	-12	-15	-16	-17	-20	-54	-68	-227	-244	101	102
29 d9 EtOAc	40	38	9	0	-5	2	3	-12	-134	-99	-28	25
29 d9 MeOH	-6	-7	0	-37	-23	-19	-65	-160	86	40	77	85
34 d1 EtOAc	30	34	-14	-16	-3	-8	-22	-52	-144	-178	-55	7
34 d1 MeOH	24	21	-11	-33	-7	-5	-107	-133	-238	-252	56	71

34 d6 EtOAc	34	30	-27	-23	-5	15	-26	-55	-48	-126	-1	30
34 d6 MeOH	27	29	-6	-16	-5	-2	-70	-76	-225	-137	97	90
34 d9 EtOAc	34	31	-12	-26	-6	2	-22	-23	-56	-94	-6	36
34 d9 MeOH	32	28	-7	-10	-10	-4	-83	-80	-245	-250	68	80
48 d1 EtOAc	-13	18	7	5	-6	9	-2	-21	-18	-48	-57	18
48 d1 MeOH	-14	-14	-92	-72	-15	-13	-128	-144	-154	-156	75	83
48 d6 EtOAc	11	-1	5	12	-6	9	-7	-31	-109	-92	-21	41
48 d6 MeOH	18	17	-34	-30	7	14	-133	-145	-148	-126	91	92
48 d9 EtOAc	28	24	21	-4	-4	-25	-3	-9	-107	-243	-49	4
48 d9 MeOH	9	8	-39	-47	4	9	-142	-142	-125	-104	93	96
50 d1 EtOAc	30	30	-31	-20	3	11	-21	-40	-182	-5	-37	17
50 d1 MeOH	7	7	-45	-54	-57	-14	-154	-142	-123	-154	58	80
50 d6 EtOAc	39	33	18	-2	-1	-2	-8	-35	-140	-192	-13	42
50 d6 MeOH	6	3	-7	-19	-10	5	-74	-88	9	12	98	99
50 d9 EtOAc	40	31	5	4	3	-12	-11	-17	-150	-134	-13	39
50 d9 MeOH	8	4	-7	-23	-14	1	-67	-98	17	28	98	99
54 d1 EtOAc	19	20	0	-10	-9	-2	-27	-34	-66	-147	-23	35
54 d1 MeOH	-4	-21	-20	-53	-76	-24	-100	-129	-85	20	79	86
54 d6 EtOAc	13	10	16	-2	-1	9	-13	-19	-127	-126	-2	39
54 d6 MeOH	7	13	-14	-13	-23	-9	-89	-125	-134	65	95	99
54 d9 EtOAc	29	30	13	-2	3	3	-1	-13	-109	-61	-26	16
54 d9 MeOH	14	19	8	-10	-48	-13	-79	-85	-179	-194	91	93
56 d1 EtOAc	33	34	-13	-29	7	-4	-45	-58	-156	-126	-4	36
56 d1 MeOH	84	91	85	79	88	81	99	97	91	98	96	98
56 d6 EtOAc	32	35	-8	-15	-1	3	-2	-13	-102	-140	-3	40
56 d6 MeOH	26	31	99	100	-35	-4	97	97	65	89	101	101
56 d9 EtOAc	31	38	1	-3	-2	-1	-1	-16	-180	-83	2	37
56 d9 MeOH	100	100	101	101	100	100	101	101	101	107	101	102
71 d1 EtOAc	34	38	14	-2	-5	-1	-4	-24	-196	-48	-38	30
71 d1 MeOH	-9	-8	-49	-53	-23	-36	-149	-151	50	24	83	86
71 d6 EtOAc	1	8	9	-3	-5	-3	-23	-36	-112	-84	3	28
71 d6 MeOH	5	4	-59	-66	-65	-20	-174	-198	-143	-153	69	89
71 d9 EtOAc	35	30	4	-7	-1	5	-25	-32	-113	-282	-18	37
71 d9 MeOH	-3	-6	-59	-63	-77	-54	-123	-127	73	-208	87	93
79 d1 EtOAc	36	36	0	-10	-3	4	-21	-36	-62	-113	-62	9
79 d1 MeOH	7	-3	-39	-38	-44	-48	-107	-80	-88	-143	78	87
79 d6 EtOAc	19	21	7	-9	-11	1	-19	-37	-39	-99	17	49
79 d6 MeOH	1	-1	-24	-61	-6	-4	-113	-135	19	-126	98	100
79 d9 EtOAc	25	18	14	5	8	-2	-1	-23	-135	-97	-3	38
79 d9 MeOH	2	2	-9	-22	1	-6	-65	-89	-21	-142	95	97
93 d1 EtOAc	29	32	-6	-21	-2	-6	-37	-21	-160	16	-23	9
93 d1 MeOH	13	7	-29	-33	-1	1	-99	-117	-195	-186	50	78
93 d6 EtOAc	31	23	-17	-14	6	9	-30	-61	-149	-128	-50	16
93 d6 MeOH	14	8	-36	-39	-10	5	-129	-150	-179	-206	71	50
93 d9 EtOAc	28	25	-23	-26	-5	10	-14	-69	-58	-39	-45	14
93 d9 MeOH	16	18	-27	-38	-12	-5	-119	-142	-223	-192	69	74

2ABFH4 d1 EtOAc	30	30	-22	-31	0	7	-25	-58	22	-68	-26	28
2ABFH4 d1 MeOH	23	21	-28	-9	-11	-12	-73	-90	-126	-148	40	63
2ABFH4 d6 EtOAc	31	30	-5	-1	-7	-5	-7	-32	-123	-158	-21	7
2ABFH4 d6 MeOH	11	9	-56	-55	-15	-14	-134	-150	-110	-111	39	73
2ABFH4 d9 EtOAc	5	38	8	5	-2	17	-6	-24	-96	-115	-12	29
2ABFH4 d9 MeOH	18	8	-46	-48	-13	-12	-138	-152	-143	-97	-86	67
2ABMF2 d1 EtOAc	14	22	9	2	1	10	-106	-86	-89	-181	-82	-44
2ABMF2 d1 MeOH	-9	9	12	11	5	7	-33	-54	-227	-167	82	83
2ABMF2 d6 EtOAc	23	32	-5	10	-11	-2	-20	-17	-25	-91	-22	-39
2ABMF2 d6 MeOH	-10	0	22	-14	-75	-47	-75	-65	-18	-84	92	89
2ABMF2 d9 EtOAc	17	28	4	21	-11	-13	-22	-10	-71	-95	-19	-36
2ABMF2 d9 MeOH	36	27	7	11	3	-29	-54	-73	-99	-116	93	87
2AB_FH4 -BHI d1 EtOAc	18	25	4	21	-10	5	-19	-4	-39	-131	-29	-21
2AB_FH4-BHI d1 MeOH	-30	8	100	99	99	99	97	97	-171	-178	-37	-24
2AB_FH4-BHI d6 EtOAc	3	-6	14	1	-45	-6	-20	-33	-66	-6	-62	-77
2AB_FH4-BHI d6 MeOH	-7	9	-14	-20	3	10	-112	-111	-103	-90	17	7
2AB_FH4-BHI d9 EtOAc	-5	24	7	18	-8	-11	-26	-22	-75	-109	-39	-53
2AB_FH4-BHI d9 MeOH	-7	14	-17	-16	31	46	-58	-76	-146	-6	16	-1033
2MF3 d1 EtOAc	19	30	-2	5	10	17	-8	-11	-66	-96	-60	-63
2MF3 d1 MeOH	-14	-3	-13	-12	-15	-9	-105	-41	77	-97	27	28
2MF3 d6 EtOAc	17	24	4	29	10	14	-13	-3	-116	-102	-84	-40
2MF3 d6 MeOH	-6	-3	-25	-28	-13	3	-107	-129	6	44	50	51
2MF3 d9 EtOAc	18	30	3	12	7	17	-22	-9	-101	-95	-54	-75
2MF3 d9 MeOH	-3	21	-18	-13	-25	-7	-71	-118	-115	-75	35	26
2MF4-BHI d1 EtOAc	29	22	22	7	6	4	-16	-24	-62	-68	-25	-22
2MF4-BHI d1 MeOH	-25	-10	-18	-36	-40	-28	-63	-90	-81	-62	67	62
2MF4-BHI d6 EtOAc	-34	-3	15	16	4	3	-11	7	0	-72	-74	-44
2MF4-BHI d6 MeOH	-22	-5	1	0	-96	-18	-51	-28	-2	-4	81	64
2MF4-BHI d9 EtOAc	-40	-14	-10	15	10	14	-2	0	-4	-95	-23	-61
2MF4-BHI d9 MeOH	-16	-9	-9	-4	-32	-17	-71	-61	70	76	67	61
2MF5 d1 EtOAc	1	35	-7	17	0	-2	-21	-9	-137	-58	-60	-49
2MF5 d1 MeOH	-26	12	-34	-25	65	77	-95	-87	-168	-30	-5	-41
2MF5 d6 EtOAc	-11	16	2	27	6	12	-33	-8	-98	-47	-76	-55
2MF5 d6 MeOH	-12	1	-11	9	17	19	-86	-83	-127	-132	-23	-18
2MF5 d9 EtOAc	20	32	12	22	-1	11	-28	-12	-75	-136	-65	-77
2MF5 d9 MeOH	-31	-7	-6	-8	40	51	-77	-87	-171	-88	6	-1

2MH2 d1 EtOAc	16	31	12	13	-22	5	-18	2	-7	-30	-33	-47
2MH2 d1 MeOH	-14	-1	-24	-26	-28	4	-96	-86	62	75	37	42
2MH2 d6 EtOAc	18	26	-1	5	-1	9	-28	-8	20	-64	-72	-47
2MH2 d6 MeOH	-15	3	-27	-21	-12	-4	-75	-71	76	-26	29	-163
2MH2 d9 EtOAc	17	27	3	7	6	11	-39	-26	-168	-107	-57	-70
2MH2 d9 MeOH	-8	-13	-25	-34	-7	4	-142	-122	65	12	20	-6
2MH4 d1 EtOAc	-23	-10	10	19	9	6	3	-6	-16	-56	-11	-35
2MH4 d1 MeOH	-17	8	-13	-6	100	13	-87	-78	-180	-196	-42	-43
2MH4 d6 EtOAc	10	9	13	15	-1	-9	-18	-11	-44	-58	-23	-18
2MH4d6 MeOH	-2	17	102	100	101	99	-31	-15	-86	-21	-37	-33
2MH4 d9 EtOAc	-2	8	15	8	6	2	-13	2	-60	-16	-57	-68
2MH4 d9 MeOH	1	4	31	99	100	99	-69	-55	-106	-84	-63	-24
Fäk1_2 d1 EtOAc	14	23	-2	1	15	9	-31	-17	-145	-131	-49	-73
Fäk1_2 d1 MeOH	-16	3	-21	-23	-10	-8	-101	-103	-96	-98	82	87
Fäk1_2 d6 EtOAc	5	17	5	-4	7	16	-11	-7	-127	-164	-56	-48
Fäk1_2 d6 MeOH	-7	12	-5	-5	-12	-17	-106	-88	-5	-10	82	60
Fäk1_2 d9 EtOAc	-18	0	2	18	4	17	-11	-23	-147	-70	-30	-12
Fäk1_2 d9 MeOH	-7	2	-19	-22	-56	-24	-135	-107	-84	-100	58	28
Wild Mid 14 d1 EtOAc	9	-3	-7	6	-3	6	-81	-55	-125	-161	30	9
Wild Mid 14 d1 MeOH	-18	2	-26	-26	-15	-26	-135	-118	-63	-101	41	56
Wild Mid 14 d6 EtOAc	6	31	4	9	1	3	-31	-7	-61	-21	-29	-72
Wild Mid 14 d6 MeOH	-6	8	-8	-3	-7	-1	-93	-118	-6	-50	18	74
Wild Mid 14 d9 EtOAc	-17	20	-7	1	12	13	-40	0	-100	-115	-41	-54
Wild Mid 14 d9 MeOH	-6	7	-8	2	-10	-4	-62	-63	89	99	-100	-64
38 d1 EtOAc	9	30	-22	100	11	30	-39	-27	-119	-27	16	-19
38 d1 MeOH	85	81	37	31	13	8	-15	-34	-84	51	41	42
38 d6 EtOAc	24	0	100	28	4	-5	-39	-35	-113	-43	31	-32
38 d6 MeOH	97	100	99	100	99	95	93	89	42	71	99	97
38 d9 EtOAc	18	7	100	-1	1	29	-8	-18	-62	0	18	-14
38 d9 MeOH	21	25	10	31	22	35	-58	-66	-72	-58	63	51
39 d1 EtOAc	25	13	14	5	-3	27	-26	-10	-100	-29	1	-17
39 d1 MeOH	15	2	-3	19	-50	8	-57	-64	-125	-43	40	19
39 d6 EtOAc	30	3	1	36	-7	1	-22	-19	-34	-79	13	-19
39 d6 MeOH	69	91	97	50	2	48	-18	-45	-57	-45	104	104
39 d9 EtOAc	19	0	15	29	8	-1	-4	-6	-47	-110	-21	-93
39 d9 MeOH	-1	-17	0	21	-73	-1	-63	-68	-81	-33	104	103
41 d1 EtOAc	37	6	-31	-9	12	-12	22	12	-93	8	13	-38
41 d1 MeOH	17	-1	98	17	-37	-1	-66	-75	40	66	67	73
41 d6 EtOAc	38	-19	100	10	14	-4	10	4	-69	-38	9	-18
41 d6 MeOH	17	-26	83	15	0	-3	81	87	83	89	105	105
41 d9 EtOAc	35	21	14	32	8	-2	6	11	-98	-32	101	68
41 d9 MeOH	11	0	32	48	-58	-26	97	97	-58	19	105	105
42 d1 EtOAc	25	-2	-3	1	-23	-38	4	20	5	-28	-32	-15

42 d1 MeOH	30	99	95	54	-13	3	-42	-56	-108	59	38	30
42 d6 EtOAc	40	11	-27	-3	5	19	15	9	-70	-14	8	-14
42 d6 MeOH	100	100	100	100	100	100	101	100	75	89	99	97
42 d9 EtOAc	38	15	-31	-5	6	9	22	14	-169	-28	-47	-47
42 d9 MeOH	54	36	28	44	100	100	-25	-1	86	70	56	52
57 d1 EtOAc	-3	-19	-13	-3	-93	-1	-25	-33	16	72	-113	-93
57 d1 MeOH	11	-12	-2	17	-105	-50	13	6	-119	-53	100	99
57 d6 EtOAc	8	-13	-24	6	-16	-46	-22	-36	-66	12	21	9
57 d6 MeOH	8	-5	-3	8	-102	-35	-68	-59	-54	-13	105	105
57 d9 EtOAc	14	-23	101	7	-63	-70	-26	-44	34	-1	-52	-75
57 d9 MeOH	3	53	62	3	-60	-14	-58	-74	-81	-42	105	105
58 d1 EtOAc	2	-32	-30	2	10	-10	13	12	-86	6	-14	-16
58 d1 MeOH	32	0	6	33	-3	-27	-18	-37	-46	-22	36	39
58 d6 EtOAc	8	-22	101	5	17	26	20	10	-56	11	0	5
58 d6 MeOH	19	5	0	13	19	3	-84	-110	-73	30	102	102
58 d9 EtOAc	-10	11	101	5	16	16	21	13	-19	2	25	-13
58 d9 MeOH	8	-7	86	5	4	12	-84	-99	56	69	102	101
76 d1 EtOAc	44	22	-25	1	11	-18	19	19	50	5	-41	-79
76 d1 MeOH	33	4	-1	33	-18	-1	-46	-62	61	68	53	40
76 d6 EtOAc	40	-5	10	7	14	5	11	4	22	-9	5	-19
76 d6 MeOH	51	57	100	100	33	28	-28	-24	-64	-73	104	104
76 d9 EtOAc	38	21	-9	15	0	18	7	0	-91	-5	-14	-17
76 d9 MeOH	38	-6	1	12	6	5	-10	29	-88	67	50	39
77 d1 EtOAc	16	-12	101	4	8	-3	9	4	-3	8	-28	-32
77 d1 MeOH	17	-9	51	59	13	9	4	-24	40	-40	58	68
77 d6 EtOAc	23	12	-23	5	7	-32	11	5	-28	24	-6	-41
77 d6 MeOH	63	30	87	96	14	3	-24	0	41	-9	56	52
77 d9 EtOAc	20	7	-25	9	10	-25	9	6	-31	37	-55	-42
77 d9 MeOH	23	3	7	24	-9	-5	-58	-72	-29	-17	37	22
88 d1 EtOAc	37	6	-8	101	5	7	-8	-18	-81	-20	-10	-39
88 d1 MeOH	39	21	16	19	5	7	-33	-75	-58	-34	73	35
88 d6 EtOAc	31	12	19	7	6	15	12	8	-68	-73	8	-29
88 d6 MeOH	24	3	-11	7	3	17	-60	-73	-92	-35	103	102
88 d9 EtOAc	7	-21	-23	14	16	25	20	11	-117	-9	15	10
88 d9 MeOH	44	20	0	18	4	6	-57	-64	-48	-19	101	100
89 d1 EtOAc	7	-29	-26	10	8	22	18	7	11	-16	-18	-17
89 d1 MeOH	39	-1	100	34	1	28	41	-1	97	96	41	8
89 d6 EtOAc	39	19	-12	6	11	5	14	-1	-35	-29	-57	-23
89 d6 MeOH	100	101	100	100	101	101	101	101	78	45	55	72
89 d9 EtOAc	38	4	-5	34	7	-2	-5	-9	-57	44	-25	-51
89 d9 MeOH	100	101	101	101	101	101	101	99	-96	-13	55	83
2FH1_PGS d1 EtOAc	0	-3	22	14	6	7	10	-1	-55	-1	-51	-31
2FH1_PGS d1 MeOH	43	10	18	54	-13	-14	-16	-46	66	40	74	64
2FH1_PGS d6 EtOAc	12	-13	-16	13	3	8	12	-16	-44	-2	-21	-13
2FH1_PGS d6 MeOH	97	89	97	96	-26	-19	58	-64	-18	17	95	89

2FH1_PGS d9 EtOAc	8	-2	-18	10	6	-16	12	-18	-49	-42	-3	-19
2FH1_PGS d9 MeOH	63	100	29	53	-29	-20	-22	-45	35	80	69	64
2FH3_PGS d1 EtOAc	13	-22	11	10	3	14	6	-24	-48	19	-49	-89
2FH3_PGS d1 MeOH	30	-12	93	99	-20	-18	-84	10	67	71	75	72
2FH3_PGS d6 EtOAc	36	9	-3	23	13	-2	-6	-33	7	-28	-41	-43
2FH3_PGS d6 MeOH	13	-11	99	98	-29	-56	-60	-6	-65	-29	-30	-65
2FH3_PGS d9 EtOAc	30	8	19	22	11	-23	-3	-55	-36	-10	-11	-26
2FH3_PGS d9 MeOH	6	-6	100	98	-39	-33	-63	-41	-31	33	-17	-66
2FM6_PGS d1 EtOAc	37	10	-2	22	-2	22	-5	-65	61	-51	1	-19
2FM6_PGS d1 MeOH	37	4	100	18	0	2	-41	-73	-123	83	52	41
2FM6_PGS d6 EtOAc	35	-2	-1	29	3	16	-7	-71	-60	-30	-35	-15
2FM6_PGS d6 MeOH	100	101	101	100	100	101	100	100	37	3	70	74
2FM6_PGS d9 EtOAc	11	25	11	101	-10	-11	-12	-62	-56	11	0	-22
2FM6_PGS d9 MeOH	42	25	95	87	101	101	29	88	53	79	57	58
2MF1_PGS d1 EtOAc	62	12	11	101	15	-8	-36	-34	-112	-356	-27	-30
2MF1_PGS d1 MeOH	83	24	-32	-22	-15	1	0	15	-173	-290	9	45
2MF1_PGS d6 EtOAc	15	19	9	-15	-3	17	-16	-39	-16	-127	-42	-34
2MF1_PGS d6 MeOH	34	4	86	26	1	-43	94	79	-10	-198	104	104
2MF1_PGS d9 EtOAc	15	18	5	-22	12	16	-35	-52	-3	-44	-51	-55
2MF1_PGS d9 MeOH	3	5	-38	64	7	-54	53	36	-79	-58	104	104
2MF6_PGS d1 EtOAc	12	10	2	-25	4	4	-41	-42	-33	-153	-19	-37
2MF6_PGS d1 MeOH	14	33	-11	-16	43	37	-56	-76	-112	-258	-96	-21
2MF6_PGS d6 EtOAc	4	21	9	-1	-13	1	0	13	-67	-242	-68	-74
2MF6_PGS d6 MeOH	99	99	89	101	99	97	100	101	-206	-208	26	25
2MF6_PGS d9 EtOAc	4	36	15	4	2	-3	8	5	-60	-81	-17	-25
2MF6_PGS d9 MeOH	99	98	99	101	100	96	95	100	38	-151	25	37
2MH1_PGS d1 EtOAc	18	25	11	-6	-3	-27	-3	0	-55	-240	-88	-87
2MH1_PGS d1 MeOH	2	10	-32	-41	-64	-65	-15	-38	-100	-314	59	66
2MH1_PGS d6 EtOAc	24	26	10	4	2	-7	2	4	77	-230	-73	-51
2MH1_PGS d6 MeOH	0	4	3	18	-13	-20	72	64	-14	-115	104	104
2MH1_PGS d9 EtOAc	25	18	15	102	5	0	-1	-7	34	-17	-59	-79
2MH1_PGS d9 MeOH	19	23	-7	73	-22	-52	-65	-68	-95	-165	104	104

Man. F. hind 1 d1 EtOAc	24	5	12	1	5	14	10	5	-52	-243	-98	-21
Man. F. hind 1 d1 MeOH	32	24	-5	-2	3	-11	-55	-81	87	-198	48	59
Man. F. hind 1 d6 EtOAc	23	24	-13	-7	0	0	7	0	16	-213	-40	-13
Man. F. hind 1 d6 MeOH	46	37	14	23	-40	-65	7	3	57	-86	99	98
Man. F. hind 1 d9 EtOAc	27	18	11	-5	6	-2	11	5	-74	-124	-77	-39
Man. F. hind 1 d9 MeOH	37	59	95	34	-96	0	-51	-65	-14	-187	92	89
Man. F. hind 2 d1 EtOAc	26	35	10	4	5	-3	9	4	-55	-81	-109	-114
Man. F. hind 2 d1 MeOH	37	36	-13	27	8	42	20	-7	-71	-246	48	45
Man. F. hind 2 d6 EtOAc	32	26	8	8	-6	-10	6	0	-63	-177	-54	-29
Man. F. hind 2 d6 MeOH	5	6	-28	-31	-23	-21	-65	-69	-75	-176	-23	27
Man. F. hind 2 d9 EtOAc	16	-14	16	4	20	12	7	-1	-62	-105	-28	-13
Man. F. hind 2 d9 MeOH	-11	-3	-39	-41	-110	-65	-64	-77	81	-84	104	103
Man. F. mid 1 d1 EtOAc	32	-5	8	13	10	5	17	5	-67	-108	-25	-70
Man. F. mid 1 d1 MeOH	19	27	-14	-3	13	6	16	-20	-76	-272	41	37
Man. F. mid 1 d6 EtOAc	23	-10	0	1	7	5	3	0	-24	-114	-74	-53
Man. F. mid 1 d6 MeOH	17	-5	13	101	-11	-3	12	38	-128	-27	33	43
Man. F. mid 1 d9 EtOAc	24	-9	-2	4	7	11	0	-5	37	-55	-41	-27
Man. F. mid 1 d9 MeOH	26	19	4	11	2	1	22	36	-108	-91	38	43
Man. F. mid 4 d1 EtOAc	26	31	8	-7	6	2	8	-8	-23	-190	-104	-32
Man. F. mid 4 d1 MeOH	9	11	-23	-20	11	3	-70	-75	-103	-258	43	43
Man. F. mid 4 d6 EtOAc	13	17	2	2	-21	-9	3	-5	-97	-71	-62	-38
Man. F. mid 4 d6 MeOH	10	-15	99	4	-15	-14	26	23	-109	-5	34	37
Man. F. mid 4 d9 EtOAc	-10	-4	14	-1	0	12	6	-3	8	-58	-38	-40
Man. F. mid 4 d9 MeOH	7	-21	10	13	-15	1	28	24	-40	-162	38	39
Man. MF4 d1 EtOAc	18	-6	101	4	11	6	15	8	-67	-85	-116	-66
Man. MF4 d1 MeOH	66	13	7	92	-25	-16	2	34	19	-10	63	66
Man. MF4 d6 EtOAc	27	-12	11	9	7	5	9	4	-47	-89	-65	-26
Man. MF4 d6 MeOH	4	8	68	99	-18	-26	-1	-18	50	21	9	-12
Man. MF4 d9 EtOAc	23	30	1	-8	11	5	-7	-1	-7	-130	-62	-41
Man. MF4 d9 MeOH	-6	-5	8	55	-35	-43	0	-7	-99	-175	100	101
Fäk1_PGS d1 EtOAc	10	27	1	-1	3	6	14	2	-118	-133	-86	-20
Fäk1_PGS d1 MeOH	25	24	-11	-30	0	-16	-47	-60	-63	-332	58	67

Fäk1_PGS d6 EtOAc	22	9	-2	-8	8	19	6	-11	-44	-65	-31	-72
Fäk1_PGS d6 MeOH	-4	-3	-35	-47	-2	-7	-78	-88	-99	-19	81	88
Fäk1_PGS d9 EtOAc	-12	-10	4	9	14	8	10	4	15	-70	-30	-35
Fäk1_PGS d9 MeOH	-13	8	-21	0	-6	-29	-59	-65	59	100	104	104
21 d1 EtOAc	30	-5	-40	2	1	-2	-39	-18	-95	-53	-18	-23
21 d1 MeOH	-4	0	-94	-49	-78	-75	-135	-90	-82	-96	59	50
21 d6 EtOAc	13	15	-22	100	3	5	1	-20	-17	2	19	20
21 d6 MeOH	11	4	-100	95	-62	-72	-105	-139	-87	-45	16	23
21 d9 EtOAc	1	8	-39	-32	0	0	-17	-117	-69	-31	5	6
21 d9 MeOH	15	1	-34	100	-28	-8	-109	-160	-56	-27	102	101
32 d1 EtOAc	25	32	-37	3	1	8	-31	-32	-169	-49	-15	-67
32 d1 MeOH	0	7	-79	-11	-18	-1	-128	-83	-111	-65	62	41
32 d6 EtOAc	10	17	-19	-22	9	2	-9	-20	-18	-1	84	92
32 d6 MeOH	19	23	-47	-39	-2	-11	-73	-141	-64	-33	102	103
32 d9 EtOAc	36	33	-32	101	-2	-3	-29	-33	-51	-32	37	34
32 d9 MeOH	-5	-5	-116	-48	-109	-11	-123	-152	-103	-50	57	52
46 d1 EtOAc	18	33	-72	-13	1	-2	-11	-50	-37	-5	30	8
46 d1 MeOH	-16	-5	-92	-52	-23	2	-130	-90	-106	-51	60	54
46 d6 EtOAc	10	14	-26	-22	1	-1	-16	-26	-26	-3	35	32
46 d6 MeOH	9	4	-76	-59	-19	-5	-73	-149	-47	-7	64	46
46 d9 EtOAc	5	11	-16	-22	1	3	-39	-28	-96	-30	21	15
46 d9 MeOH	7	3	-65	-23	-99	-8	-108	-142	-78	-41	51	42
53 d1 EtOAc	27	-6	-35	-51	-4	-7	-32	-81	-79	-21	61	43
53 d1 MeOH	22	28	-55	-6	-7	-9	-121	-45	-71	-38	39	22
53 d6 EtOAc	1	15	-31	-17	3	0	-9	-26	-34	-6	42	43
53 d6 MeOH	-4	-7	-97	-58	-41	-18	-118	-159	-80	-61	63	54
53 d9 EtOAc	12	32	-51	5	-4	-3	-9	-27	-33	-5	38	44
53 d9 MeOH	12	3	94	-56	-19	-9	-122	-159	-63	-32	54	40
64 d1 EtOAc	31	32	-26	-8	-9	-7	-48	-37	-86	-39	37	34
64 d1 MeOH	23	9	-89	76	-9	-15	-88	-66	-111	-74	-13	25
64 d6 EtOAc	7	12	-13	-30	-1	2	-36	-27	-61	-15	38	46
64 d6 MeOH	10	9	99	-39	-7	-8	-123	-118	-83	-38	47	25
64 d9 EtOAc	23	32	102	-1	-6	-4	-53	-35	-50	-31	22	37
64 d9 MeOH	-5	2	-50	-34	-12	-24	-154	-46	-102	-70	63	45
69 d1 EtOAc	32	3	-26	-48	-3	2	-28	-22	-63	-33	48	42
69 d1 MeOH	16	-9	-91	-65	-2	-26	-113	-164	-52	-42	72	40
69 d6 EtOAc	6	31	-74	-3	4	-8	-9	-53	-61	0	57	43
69 d6 MeOH	-4	11	-89	93	-18	-31	-126	-155	-81	-44	56	46
69 d9 EtOAc	7	23	-59	-19	4	3	-3	-37	-14	9	43	27
69 d9 MeOH	-6	7	-97	6	-111	-18	-130	95	-62	-39	102	103
72 d1 EtOAc	25	-1	-29	-38	-7	3	-43	-22	-74	-41	-2	19
72 d1 MeOH	21	13	100	101	16	15	-82	-42	-66	-13	61	45
72 d6 EtOAc	4	24	102	-24	-1	-6	-21	-27	-50	-32	37	32
72 d6 MeOH	5	3	-83	90	-22	-27	-123	-101	-74	-43	102	103
72 d9 EtOAc	30	19	-26	-34	-8	-5	-40	-20	-40	-34	56	59

72 d9 MeOH	16	2	-116	-51	-15	-22	-63	-123	-56	-34	58	44
98 d1 EtOAc	31	34	-32	-33	-10	-13	-30	-32	-67	-22	53	50
98 d1 MeOH	0	-2	-51	-66	7	-8	-108	-112	-55	-34	42	19
98 d6 EtOAc	31	30	102	-28	-9	-6	-26	-33	-55	-39	41	45
98 d6 MeOH	4	1	-63	-55	-16	-21	-116	-158	-63	-30	47	32
98 d9 EtOAc	-11	28	-58	-32	-2	1	-4	-46	-1	-9	17	16
98 d9 MeOH	31	15	-66	-33	-26	15	-108	-65	-44	-57	57	31
AB5 d1 EtOAc	25	8	102	-31	-8	0	-33	-27	-70	-35	42	41
AB5 d1 MeOH	30	2	-49	-52	-10	-8	-108	-110	-42	-34	27	36
AB5 d6 EtOAc	27	33	-20	-40	-8	-5	-32	-26	-60	-33	40	43
AB5 d6 MeOH	10	8	-63	-38	-12	-11	-76	-103	-27	-5	65	56
AB5 d9 EtOAc	35	18	102	-23	-4	-6	-24	-42	-31	-18	46	32
AB5 d9 MeOH	15	9	-60	-27	-13	-17	-93	-104	-30	-9	63	58
2AB_MF1 d1 EtOAc	29	15	101	-33	-8	-9	-30	-32	-60	-40	52	22
2AB_MF1 d1 MeOH	1	14	-95	-43	-15	-5	-114	-70	-89	-69	40	61
2AB_MF1 d6 EtOAc	21	29	-54	-3	-8	5	-34	-59	-64	-45	101	103
2AB_MF1 d6 MeOH	9	17	-58	102	21	6	-59	-40	-28	-52	99	102
2AB_MF1 d9 EtOAc	22	21	-48	102	-4	0	-26	-38	-89	-54	36	36
2AB_MF1 d9 MeOH	1	12	-88	-49	-19	11	-106	-62	-80	-57	64	51
Wild_Anal_6 d1 EtOAc	24	31	102	-14	-8	0	-24	-33	-74	-35	28	30
Wild_Anal_6 d1 MeOH	12	1	-52	-29	-5	-13	-55	-141	-38	-16	-17	-38
Wild_Anal_6 d6 EtOAc	2	27	-59	101	3	-3	-14	-42	-39	-10	32	27
Wild_Anal_6 d6 MeOH	15	11	-45	101	0	-9	-84	-133	-74	-51	15	-45
Wild_Anal_6 d9 EtOAc	6	26	-42	-29	2	13	-26	-24	-38	-30	49	34
Wild_Anal_6 d9 MeOH	-1	13	-78	-31	0	-13	-123	-142	-79	-61	47	-6
2AB_MF3 d1 EtOAc	14	18	-63	-9	-1	-4	-64	-71	-81	-57	59	48
2AB_MF3 d1 MeOH	8	16	-43	-33	-14	5	-75	-26	-40	-32	52	7
2AB_MF3 d6 EtOAc	10	14	-58	-30	14	9	-44	-37	-36	-34	102	102
2AB_MF3 d6 MeOH	19	-12	-72	-66	-6	-10	-71	-141	-11	10	102	103
2AB_MF3 d9 EtOAc	15	17	-47	-1	7	2	-26	-34	-37	-67	44	35
2AB_MF3 d9 MeOH	-10	7	-96	-29	-31	-5	-121	-124	-98	-81	63	51
H10 d1 EtOAc	5	19	-83	4	3	16	-28	-61	-18	-24	26	17
H10 d1 MeOH	17	2	-23	-54	12	4	-93	-115	-102	-64	-28	-52
H10 d6 EtOAc	10	34	-29	-3	-5	23	-56	-42	-57	-57	18	-8
H10 d6 MeOH	9	-1	96	-44	-8	1	-96	-122	-110	-64	45	29
H10 d9 EtOAc	16	36	-22	102	-2	20	-31	-70	-43	-66	31	21
H10 d9 MeOH	2	-4	100	-34	-4	-4	-111	-99	-92	-69	-21	-67
B02 d1 EtOAc	36	15	-16	101	0	3	-56	-33	-6	-5	26	22

B02 d1 MeOH	27	-8	-62	-344	-50	-16	-136	-106	-52	-45	-15	-30
B02 d6 EtOAc	10	7	99	101	-23	6	-7	-20	-10	1	12	8
B02 d6 MeOH	14	-4	-64	-334	-79	-74	-136	-156	-30	-34	-39	-19
B02 d9 EtOAc	7	-1	-29	-305	-1	-55	-30	-129	-35	-26	4	-8
B02 d9 MeOH	16	3	89	99	-35	-11	-155	-164	-37	-35	13	30
C11 d1 EtOAc	31	19	-14	-215	0	6	-33	-23	1	-1	39	39
C11 d1 MeOH	8	-3	89	-237	-21	-2	-159	-139	-48	-43	20	21
C11 d6 EtOAc	6	14	9	-274	-19	-6	-25	-23	8	15	5	8
C11 d6 MeOH	8	23	98	-355	-1	-7	-86	-147	-49	-49	99	99
C11 d9 EtOAc	10	-5	-7	103	-1	1	-17	-28	-11	-3	13	33
C11 d9 MeOH	0	13	71	-362	-44	-16	-156	-163	-24	-36	0	-7
E02 d1 EtOAc	0	2	-39	-220	2	-1	-13	-52	-17	-1	32	32
E02 d1 MeOH	11	-10	-56	-373	-10	-5	-144	-98	-56	-50	64	74
E02 d6 EtOAc	10	-5	-30	102	-1	-1	-15	-22	-11	0	27	29
E02 d6 MeOH	15	-3	-57	-368	-30	-62	-87	-164	-43	-32	45	-24
E02 d9 EtOAc	14	-5	-33	-265	-2	2	-35	-26	-10	-2	25	25
E02 d9 MeOH	15	-4	-37	-250	-28	-2	-145	-165	-43	-36	-19	-4
F05 d1 MeOH	39	-11	-2	-383	-7	2	-44	-88	-56	-51	-20	-26
F05 d1 EtOAc	-2	28	36	103	3	-5	-146	-52	-17	-8	27	30
F05 d6 EtOAc	1	5	101	-260	-2	8	-14	-27	6	1	18	23
F05 d6 MeOH	0	-14	61	-428	-55	-24	-146	-175	-11	-12	61	65
F05 d9 EtOAc	4	-1	-39	104	-3	-4	-40	-22	-4	5	30	30
F05 d9 MeOH	18	-5	95	-375	-21	-9	-151	-172	-30	-32	53	43
3FM1 d1 EtOAc	37	-19	-8	-194	-10	-3	-57	-49	-14	3	36	33
3FM1 d1 MeOH	9	0	-62	65	-15	-22	-113	-161	-49	-42	99	99
3FM1 d6 EtOAc	9	26	101	104	1	1	-45	-29	-5	-10	27	29
3FM1 d6 MeOH	37	2	-45	-309	-6	-11	-134	-108	-44	-37	60	60
3FM1 d9 EtOAc	30	-5	-22	-321	-9	-3	-67	-33	-15	0	35	37
3FM1 d9 MeOH	-6	4	37	-327	-18	-23	-186	-106	-57	-44	102	101
3MA3 d1 EtOAc	39	-11	-4	-333	-8	10	-42	-23	11	10	44	41
3MA3 d1 MeOH	33	-19	-57	-407	-6	-64	-140	-189	-34	-24	56	32
3MA3 d6 EtOAc	25	4	-31	-204	8	-5	-23	-48	-6	6	45	35
3MA3 d6 MeOH	8	-50	-57	60	-28	-34	-151	-160	-10	-7	102	102
3MA3 d9 EtOAc	15	19	-31	-218	9	0	-5	-40	-30	-17	101	101
3MA3 d9 MeOH	2	3	-64	-262	-75	-37	-161	95	22	1	102	102
3FA1 d1 EtOAc	39	-6	-9	106	-8	10	-47	-10	20	22	23	33
3FA1 d1 MeOH	11	-15	89	-262	24	12	-72	-19	4	-11	41	44
3FA1 d6 EtOAc	5	24	-34	106	0	3	-14	-27	1	-5	22	40
3FA1 d6 MeOH	12	0	-66	-380	-28	-25	-151	-98	-76	-71	85	72
3FA1 d9 EtOAc	7	-6	101	-286	-11	3	-42	-9	6	2	27	42
3FA1 d9 MeOH	14	-4	-54	101	-23	-22	-77	-136	-54	-52	101	101
3MA5 d1 EtOAc	39	-1	-7	105	-12	-7	-37	-24	-7	15	34	42
3MA5 d1 MeOH	12	0	-50	-348	4	-47	-117	-143	-71	-68	-6	-9
3MA5 d6 EtOAc	9	5	-15	105	-7	3	-35	-39	-3	8	38	40
3MA5 d6 MeOH	16	-2	-48	-385	-22	-40	-122	-181	-79	-79	94	96
3MA5 d9 EtOAc	5	-5	-39	105	12	12	-17	-13	-3	-1	4	17
3MA5 d9 MeOH	34	-10	-19	104	-6	7	-78	-52	-17	-22	62	63

3FA2 d1 EtOAc	30	-4	-10	106	-10	2	-39	-26	-7	3	31	28
3FA2 d1 MeOH	26	-2	-48	102	-19	-12	-97	-98	-65	-59	12	-7
3FA2 d6 EtOAc	36	4	-18	-310	-11	3	-36	-32	1	6	43	48
3FA2 d6 MeOH	28	7	-37	102	-18	5	-99	-110	-39	-50	41	20
3FA2 d9 EtOAc	20	22	102	105	-8	1	-28	-19	6	9	40	45
3FA2 d9 MeOH	20	7	-35	103	-14	-8	-120	-111	-62	-51	43	28
3MH2 d1 EtOAc	42	1	-13	-310	-8	6	-32	-17	11	21	37	24
3MH2 d1 MeOH	7	10	-46	101	-24	8	-105	-73	-56	-38	-22	-1
3MH2 d6 EtOAc	32	26	-25	-172	-5	2	-43	-58	-14	-7	17	27
3MH2 d6 MeOH	12	-6	-27	-323	13	11	-50	-25	-8	-19	27	42
3MH2 d9 EtOAc	32	24	-17	-286	-4	-4	-29	-49	-17	-35	43	43
3MH2 d9 MeOH	-4	8	-66	103	-31	15	-132	-67	-49	-56	43	36
3FA1.1 d1 EtOAc	33	25	-13	-236	-13	-2	-46	-39	-19	-20	97	46
3FA1.1 d1 MeOH	26	-12	-32	-310	-3	4	-66	-155	-50	-41	73	50
3FA1.1 d6 EtOAc	11	21	-30	-271	10	6	-14	-42	-23	1	33	21
3FA1.1 d6 MeOH	29	-3	97	100	4	1	-118	-138	-36	-33	29	32
3FA1.1 d9 EtOAc	-2	6	-39	106	2	4	-37	-24	-2	-9	29	15
3FA1.1 d9 MeOH	6	7	96	-283	0	12	-152	-141	-45	-34	101	101
3MH1 d1 EtOAc	24	-6	-34	-264	1	-7	-66	-83	-30	-14	22	30
3MH1 d1 MeOH	-4	-3	-13	-330	22	18	-99	-23	-11	-36	5	15
3MH1 d6 EtOAc	12	-2	102	-261	-6	-1	-50	-33	-8	-34	15	31
3MH1 d6 MeOH	22	-15	100	-385	-29	-41	-81	-156	-32	-43	101	101
3MH1 d9 EtOAc	11	18	-30	-259	5	-6	-31	-40	-33	-34	36	35
3MH1 d9 MeOH	0	13	-78	-280	-28	-18	-147	-144	-29	-35	101	101
3MH3 d1 EtOAc	5	-6	101	-281	17	5	-32	-31	-20	-25	14	22
3MH3 d1 MeOH	40	-12	-18	-366	15	5	-122	-130	-33	-26	27	32
3MH3 d6 EtOAc	6	13	-23	-242	-17	2	-53	-29	-59	-30	20	16
3MH3 d6 MeOH	16	-8	-63	-351	-6	-7	-126	-103	-19	-23	45	26
3MH3 d9 EtOAc	10	24	102	-239	12	-9	-26	-33	-61	-49	13	35
3MH3 d9 MeOH	9	-10	83	-363	-9	-7	-142	-104	-32	-17	10	-5

-

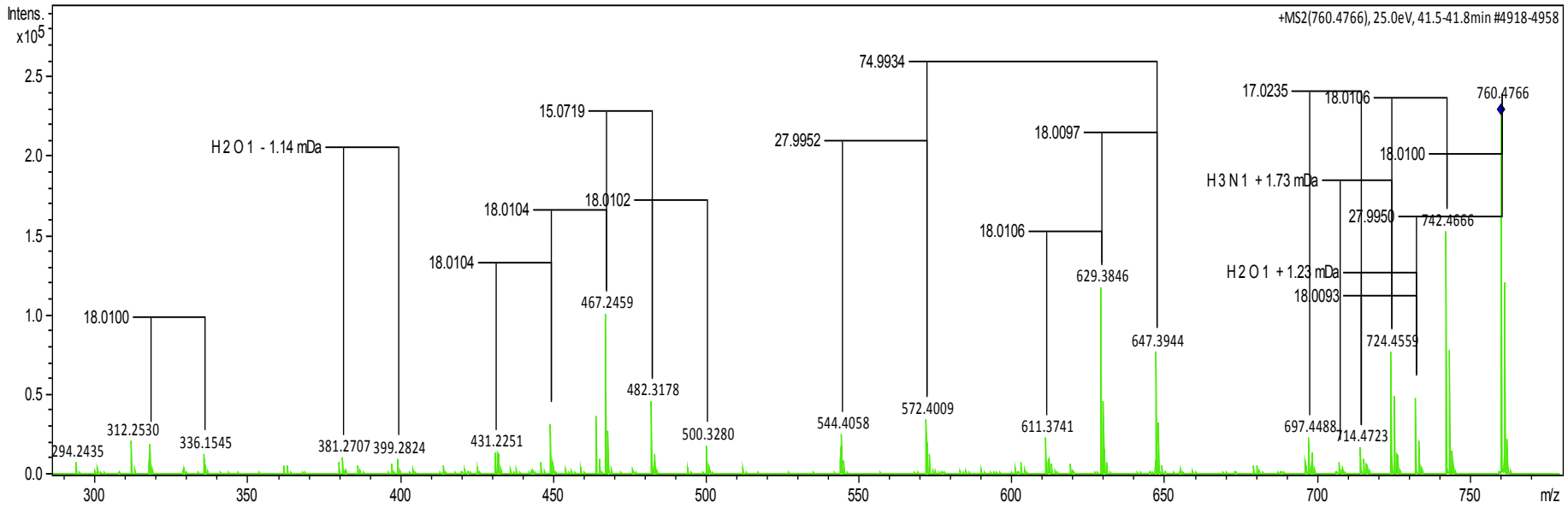


Figure S1: fragmentation pattern of the pseudomolecular ion m/z 760.4677 in the MeOH crude extract of strain 2MH3-2

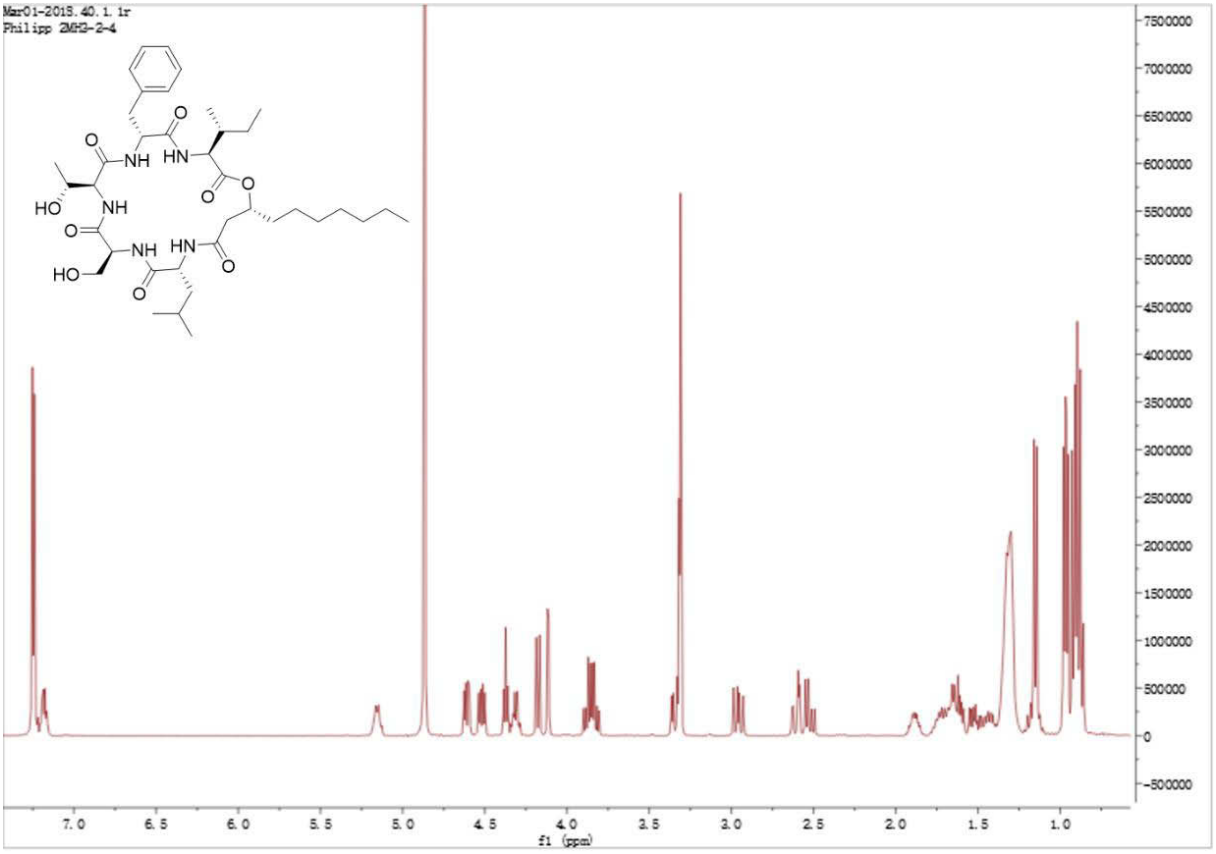


Figure S1: ^{1}H NMR (400 MHz, CH_3OH-d_4,) spectrum of serrawettin W2

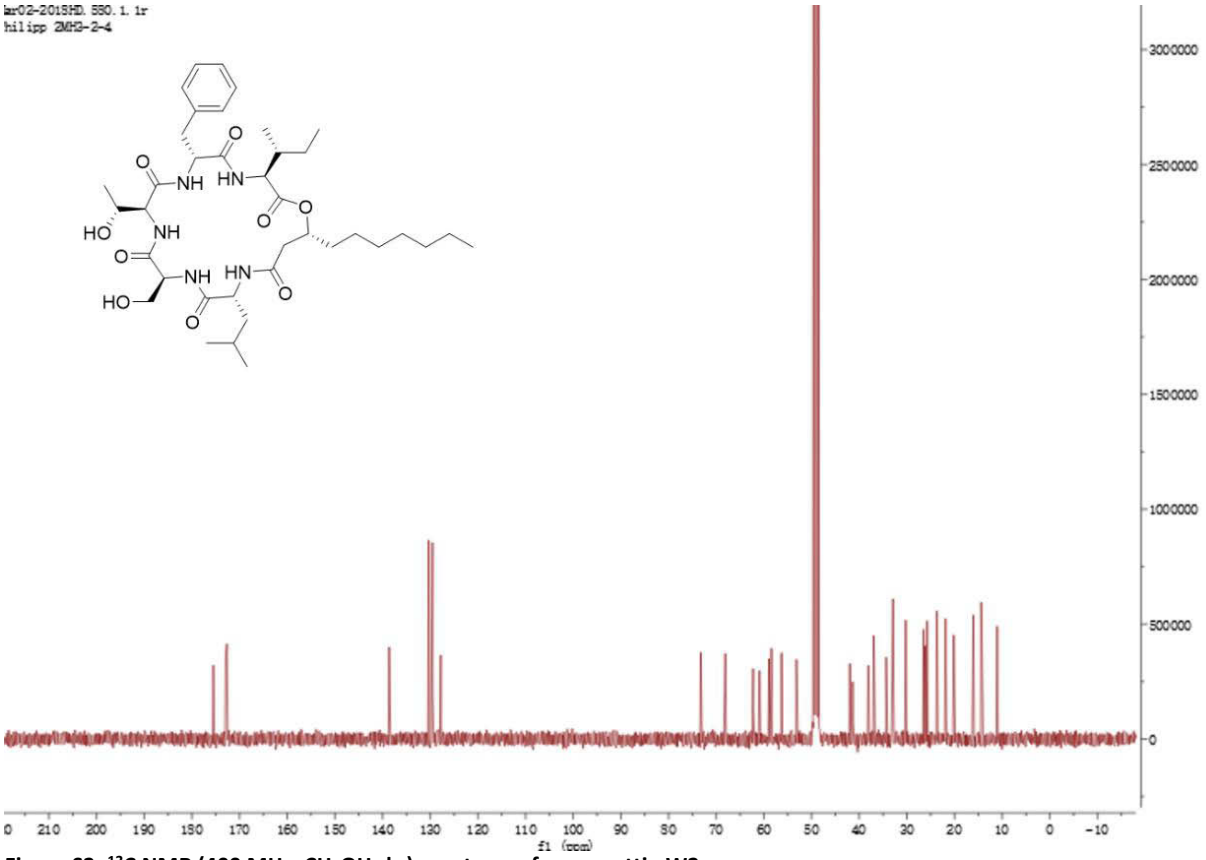


Figure S2: ^{13}C NMR (400 MHz, CH_3OH-d_4,) spectrum of serrawettin W2

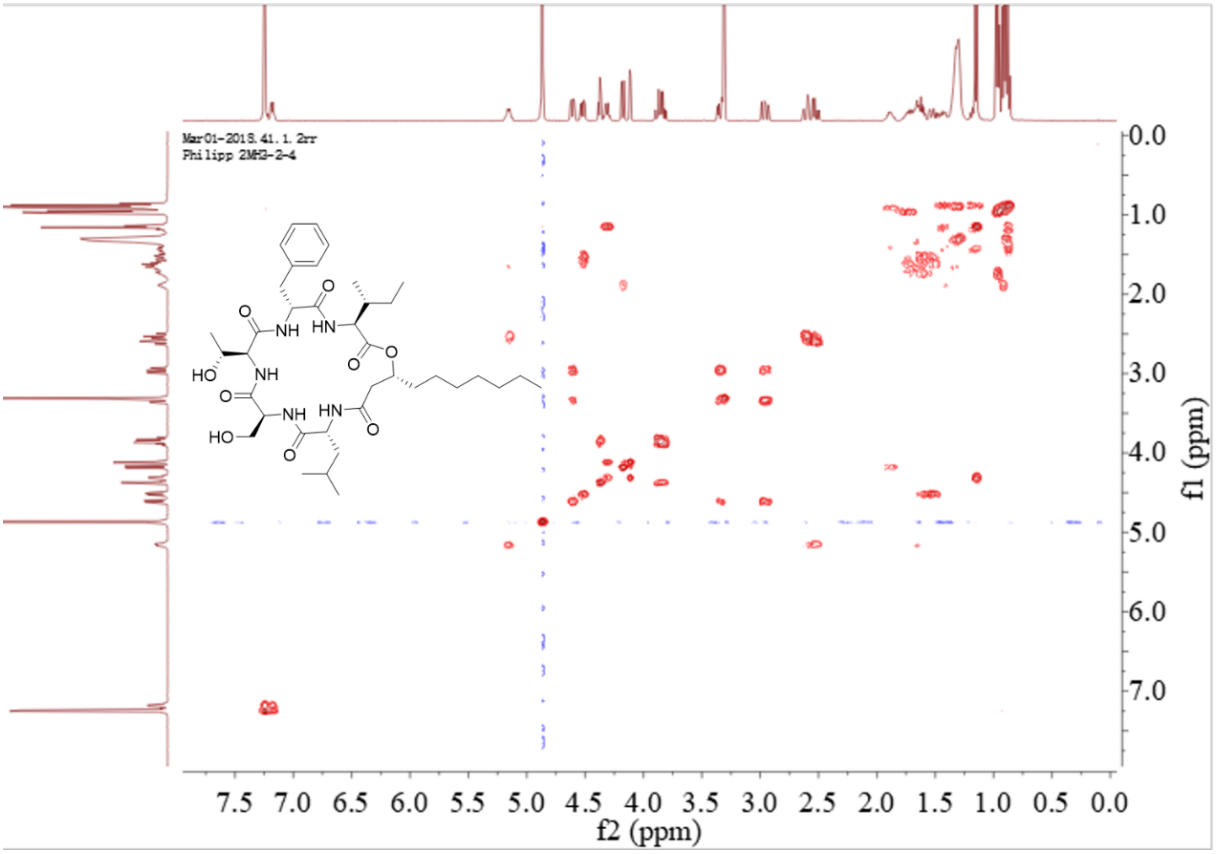


Figure S3: COSY spectrum of serrawettin W2

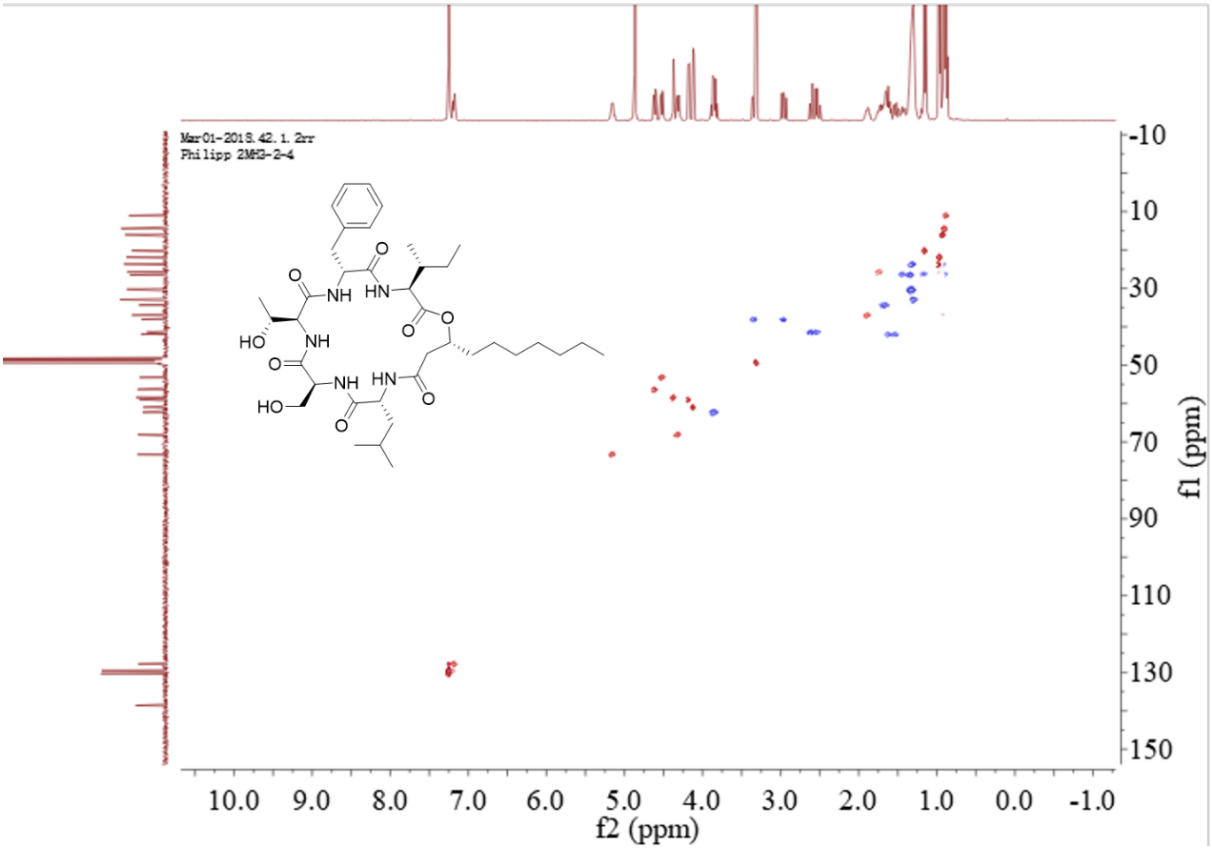


Figure S4: HSQC spectrum of serrawettin W2

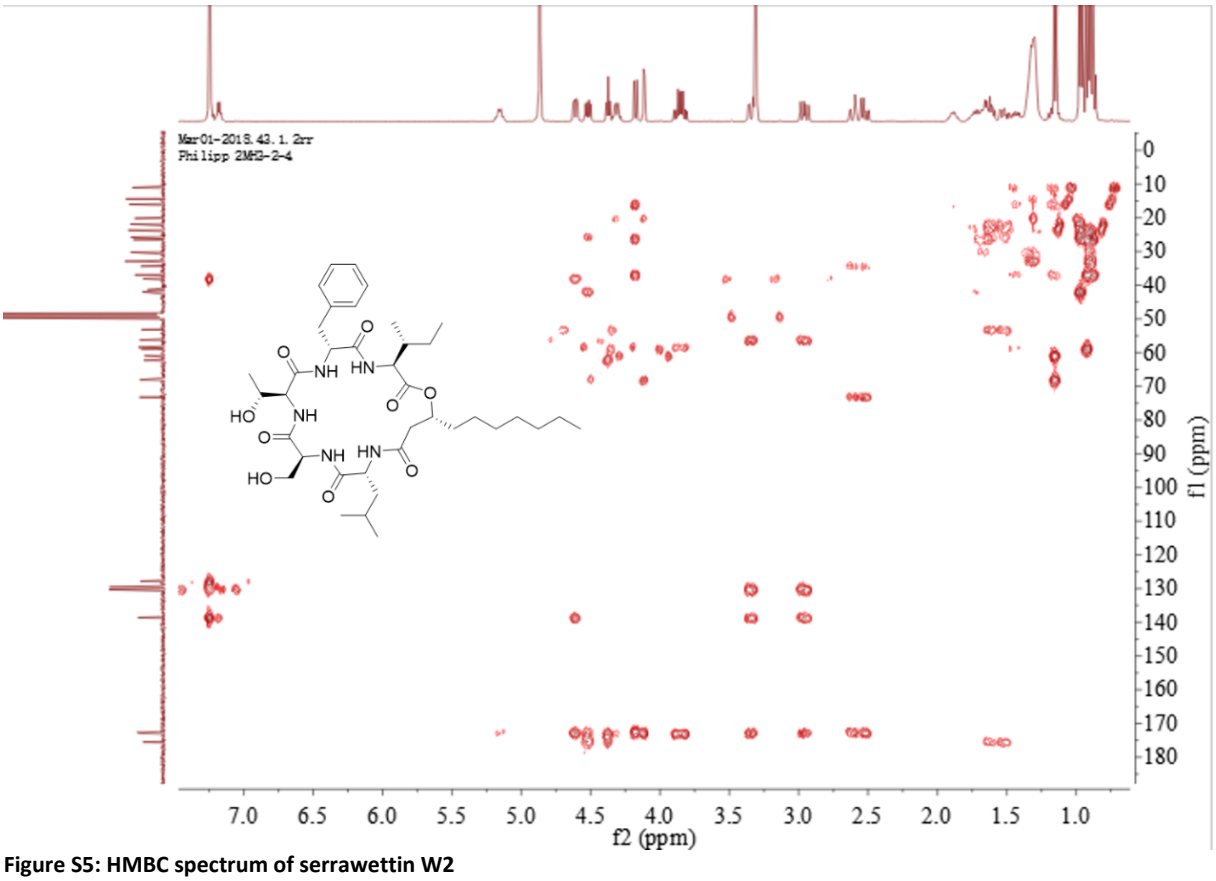


Figure S5: HMBC spectrum of serrawettin W2

Danksagung

Hiermit möchte ich mich bei allen Personen bedanken, die diese Arbeit ermöglicht und zu Entstehung beigetragen haben.

Dabei gilt mein Dank zu aller erst Herrn Prof. Andreas Vilcinskas für die Vergabe dieses interessanten Projekts, die Bereitstellung sämtlicher Forschungsmöglichkeiten am Fraunhofer IME-BR und nicht zu Letzt dem Vertrauen in meine Arbeit. Prof. Thomas Wilke danke ich für die Übernahme des Gutachtens für den Fachbereich 08 der Justus-Liebig Universität. Weiterhin möchte ich mich bei Prof. Till Schäberle für die Unterstützung durch Rat und Equipment bedanken, ohne welche die letzten praktischen Monate dieser Arbeit deutlich weniger erfolgreich ausgefallen wären.

Mein weiterer Dank gilt Dr. Thomas Degenkolb, der sowohl bei der Bearbeitung der chemisch, analytischen Fragestellungen, als auch bei der Korrektur von Manuskripten und dieser Arbeit unermüdlich mitgearbeitet hat.

Weiterhin bedanke ich mich bei Dr. Yang Liu und dem Rest unseres „Analytik Teams“ für ihre Unterstützung bei LC/MS, MS/MS, und NMR Experimenten. Nicht zu vergessen sind dabei Dr. Benedikt Leis und Kirsten Bommersheim, welche die Durchführung der Bioaktivitätstests betreut haben. Ohne euch wäre einiges nicht möglich gewesen. Auch möchte ich mich bei Dr. Henrike Schmidtberg für die Bereitstellung des fantastisch grafisch umgesetzten *Nicrophorus* Lebenszyklus bedanken.

Ich bedanke mich bei all meinen Kolleginnen und Kollegen der Insektenbiotechnologie Gruppe. Ein besonderer Dank gilt dabei Rolf Hirsch, der von Anfang an mich in unserer Sanofi-Fraunhofer Gruppe stark unterstützt hat und sich darüber hinaus zu einem guten Freund entwickelt hat. Außerdem möchte ich mich bei Tanja und Angela für ihre Unterstützung bei im Labor bedanken. Weiterhin danke ich meinen ehemaligen Kollegen Zdeněk und Andre, sowie den aktuellen: Anne, Lea, Miriam, Hendrich, Alica, John, Nils B. und Silke. Ihr ward/seid ein Teil unseres tollen Teams. Ohne euch wäre die Arbeitsatmosphäre nicht die Gleiche, viele Diskussionen bei weitem nicht so anregend und das ein oder andere Feierabendbier nicht so schmackhaft gewesen.

Zu Letzt bedanke ich mich bei meinen Eltern und meinen Freunden, die immer für mich zu da waren und auf deren Unterstützung ich zu jeder Zeit zählen konnte. Meiner Freundin Ilka danke ich für den Rückhalt, die Unterstützung und das Verständnis in jeder Situation, ohne dich wäre so manche Situation nicht gemeistert worden.

Vielen Dank!

Erklärung

Ich erkläre:

Ich, Philipp Heise (geb. 05.02.1989 in Mühlhausen/Thüringen), habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.

Gießen, den 29.05.2019