The influence of bacterial diet on behavior, metabolism and development in *Pristionchus pacificus*

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Nermin Akduman

aus Bayat/Türkei

Tübingen
2019

Tag der mündlichen Qualifikation: 05.12.19
Dekan: Prof. Dr. Wolfgang Rosenstiel
1. Berichterstatter: Prof. Dr. Ralf J. Sommer
2. Berichterstatter: Prof. Dr. Karl Forchhammer

Table of Contents

ABSTRACT

Multicellular organisms exist in intimate association with other species, including bacterial communities generally referred to as their microbiome. Microbiota can influence their host traits through communalistic, symbiotic or pathogenic relationships. The nematode *Caenorhabditis elegans* provides a powerful, genetically amenable system to study interactions between host, microbiome, and their environment. The satellite model nematode *Pristionchus pacificus* has been extensively used for evolutionary, ecological and developmental studies as a comparison to *C. elegans*. Yet *P. pacificus* has a distinct ecology, where it forms necromonic relationship with scarab beetles, and can develop two different mouth form phenotypes: either a narrow stenostomatous (St) mouth with one tooth, or a wide eurystomatous (Eu) mouth with two teeth. Eu mouth-formed nematodes feed on bacteria but can also predate on nematodes other than their own strains. In this context, *P. pacificus* provides a unique system to study microbiota influence on host traits such as development, behavior and physiology. Aiming to identify bacteria that modulate *P. pacificus* life-history traits, I isolated and identified 136 *P. pacificus* associated bacterial species from their natural environmental. I then assessed those bacteria as being pathogenic or commensal to *P. pacificus.* I used chemotaxis assays to demonstrate that *P. pacificus* prefers its natural bacteria to its long-term laboratory food source *E.coli* OP50. Further, I studied bacterial influence on *P. pacificus* life history traits, focusing on commensal bacteria isolates. Interestingly, my results showed a clear bacterial influence on *P. pacificus* predatory feeding behavior. Especially, *Novosphingobium* ssp. strains enhanced the number of predatory events such as bites and kills. However, *P. pacificus* predators did not increase their feeding frequency, indicating surplus killing behavior, a phenomenon observed with other predators. To determine the genetic and molecular basis of this effect on behavior, I created transgenic lines that would allow me to observe gene expression changes depending on the bacterial diet. Through a series of experiments that comprise the bulk of my thesis, I found that *Novosphingobium*-produced vitamin B12 enhances predatory feeding behavior in *P. pacificus* and accelerates developmental speed. The vitamin B12-mediated effect on development also extended to other species, including some parasitic nematodes, suggesting vitamin B12 is a conserved molecular catalyst for development.

ZUSAMMENFASSUNG

Alle Lebewesen existieren in enger Assoziation mit einer Vielzahl von anderen Organismen, wobei besonders Bakteriengemeinschaften (Mikrobiome) eine wichtige Rolle spielen. So übersteigt z. B. beim Menschen die Menge der den Körper besiedelnden Mikroorganismen die Anzahl der körpereigenen Zellen bei Weitem. Die Wechselwirkungen zwischen Mikrobiom und Wirt können symbiotischer, pathogener, oder kommensalisher Art sein. Der Fadenwurm *Caenorhabditis elegans* ist ein hervorragendes Modellsystem, um spezifische Interaktionen zwischen Wirt, Mikroorganismen und Umwelt auf der funktionellen und insbesondere auch der genetischen Ebene zu untersuchen. Zusätzlich steht mit der verwandten Nematodenart *Pristionchus pacificus* ein zweites Modell zur Verfügung, das es ermöglicht, vergleichende Studien durchzuführen. Während *C. elegans* ein reiner Bakterienfresser ist, hat sich bei *P. pacificus* und nahverwandten Arten eine fakultativ räuberische Lebensweise mit einem Mundformdimorphismus entwickelt. Je nach Umwelteinflüssen bilden Jungtiere eine von zwei möglichen Mundformen aus. Während die enge, stenostomate (St) Mundform mit einem Zahn lediglich Bakterien als Nahrungsquelle zulässt, umfasst das Nahrungsspektrum der Individuen mit der weiten, eurystomaten (Eu) Mundform, die über zwei Zähne verfügt, neben Bakterien auch andere Würmer, die sie mit Bissen attackieren. Ziel meiner Arbeit war es, den Einfluss von verschiedenen Bakterien auf die Entwicklung und das Fressverhalten der Nematoden zu untersuchen. Dazu habe ich 136 Bakterienarten aus dem natürlichen Lebensumfeld der Würmer isoliert, ihre Wirkung auf die Würmer getestet und geeignete nicht-pathogene Arten für die Verhaltensexperimente ausgewählt. Die Analyse des räuberischen Verhaltens der Würmer (Beißen, Töten durch Beißen, Fressen) auf verschiedenen Bakterien ergab deutliche Unterschiede, besonders bezüglich der Tötung von Beutetieren (*C. elegans*). Dabei führten verschiedene Stämme der Bakteriengattung *Novosphingobium* zu einer starken Erhöhung der Rate an Bissen und Tötungen, jedoch ohne dass mehr Kadaver gefressen wurden (surplus killing). Um die molekulare Basis dieses veränderten Verhaltens besser zu verstehen, habe ich transgene *P. pacificus* Stämme hergestellt, bei denen sich der Einfluss unterschiedlicher mikrobieller Nahrung auf der Genexpressions-, bzw. Fluoreszenzreporterebene widerspiegelte. Weiterführende Experimente, einschließlich Transposon-Mutagenese, Supplementierung und gezielten Knock-outs, haben gezeigt, dass von *Novosphingobium* gebildetes Vitamin B12 für die Verstärkung des räuberischen Verhaltens in *P. pacificus* verantwortlich ist und ebenfalls die Entwicklungsgeschwindigkeit erhöht. Letzteres konnte auch bei anderen Nematodenspecies nachgewiesen werden. Es handelt sich beim bakteriellen Vitamin B12 also um ein Schlüsselmetabolit, dass nematodenübergreifend eine wichtige entwicklungsbiologische Rolle spielt.

List of publications

1. Culture-based analysis of *Pristionchus*-associated microbiota from beetles and figs for studying nematode-bacterial interactions
Nermin Akduman, Christian Rödelsperger, Ralf J. Sommer
PLoS ONE. 13 (2018), doi:10.1371/journal.pone.0198018.(103)

2. Bacterial derived vitamin B12 enhances predatory behaviors in nematodes
Nermin Akduman, James W. Lightfoot, Waltraud Röseler, Hanh Witte, Wen-Sui Lo, Christian Rödelsperger, Ralf J. Sommer submitted for publication (2019) (104)
doi: https://doi.org/10.1101/797803

1. INTRODUCTION

1.1. Host-microbe interactions and their significance

Microbes are considered to be the dominant life form on Earth, with contemporary estimates predicting the number of microbial cells at 10^{30} and with the first microbes inhabiting the Earth approximately 2.5 billion years ago (1). Bacteria are found in every ecological niche including typical soil and water environments, as well as extreme environments, such as deep-sea hydrothermal vents, glacier ice, volcanoes and waters with high salt content (2). Bacteria are essential for life on Earth, playing key roles in nutrient sequestration and recycling, and provide the largest reservoir of carbon, nitrogen, and phosphorus on the planet. Moreover, their diverse metabolism allows them to be successful as both free living and symbiotic microorganisms. Organisms exist in intimate association with other species, especially with microbial communities. More than a century ago, Robert Koch, one of the main founders of modern bacteriology, identified microbes as the specific causative agents of infectious diseases such as tuberculosis, cholera and anthrax. For his research on tuberculosis he won the Nobel Prize in Physiology or Medicine in 1905. Around the same time, another Nobel laureate Ilya Mechnikov, "the father of natural immunity", addressed that some bacteria can be beneficial for human health and he proposed that 'lactic-acid bacteria' can prolong human life. Since then, tremendous amount of research has been carried out and diverse interactions between host and microbes have been discovered. Host–microbe interactions are as diverse as the organisms involved: these bacterial communities can be commensal, mutualistic or pathogenic to the host.

1.1.1. Host-microbe interactions: Mutualism

In mutualistic interactions, both species benefit from the interaction without harming each other. Host-bacteria mutualism is ubiquitous and there are numerous examples of these types of interactions in nature. For instance, the human gastrointestinal track is widely colonized by large numbers of bacteria cells (10-fold more than the total number of human cells) that influence the host immune system development, nutrient metabolism, physiology, and the defense against colonization by opportunistic pathogens (3). For humans, one classic mutualistic association is the lactic acid bacteria that live on the vaginal epithelium in women. The bacteria are provided with a habitat of constant temperature and supply of nutrients (glycogen) in exchange for the production of lactic acid, which protects the vagina from colonization and diseases caused by yeast and other potentially harmful microbes (4). Mutualism between insect-pathogenic bacteria of the genus *Photorhabdus* and

their insect-parasitic nematode partner *Heterorhabditis* is another fascinating example of this type of interaction. The nematode-bacteria association allows them to infect, kill and reproduce within an insect host. *Photorhabdus* colonizes the gut of infective nematode juveniles, which provides protection prior to infection. Post infection, *Photorhabdus* reproduce and kills the insect host and prepares conditions that propagate nematode development (5). Increasing interest in mutualistic bacteria research continues to contribute our existing knowledge on mutualistic interactions.

1.1.2. Host-microbe interactions: Commensalism

Commensalism is described as an interaction where one partner benefits from the interaction while the other is neither harmed nor benefited. Most of the human microbiome is considered to represent commensals, which are present on body surfaces covered by epithelial cells and are exposed to the external environment (gastrointestinal and respiratory tract, vagina, skin, etc.). Commensal bacterial communities have co-evolved within their host to survive in the host microenvironment. Co-evolution allows diverse commensal bacterial species to compete with pathogenic bacteria to reduce the ability of invasive pathogens that can establish infection and cause damage to host cells and drive disease (6). Moreover, most of the commensal bacteria carefully stimulate host defense mechanisms to inhibit expansion of invasive pathogens and preserve the environmental niche for non-invasive commensal species. For instance, *Bifidobacteria spp*. produces abundant amounts of short chain fatty acids, which inhibit the growth of enteropathogenic bacteria such as *E. coli* or *C. rodentium* (7). However, under specific conditions such as in a host with a weakened immune system, an altered microbiota or breached integumentary barriers, commensal bacteria can convert to opportunistic pathogens, which cause some of the most common infectious diseases. *Staphylococcus aureus*, extra-intestinal pathogenic *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus pneumonia* are some of the well-studied opportunistic human pathogens (8). Furthermore, commensal bacteria studies have shown that the relationship between host and commensal bacteria is highly complex and commensal bacteria have a considerable impact on the host physiology, immune system and development. Therefore, the regulation of commensal bacteria offers the possibility to strengthen the host immune system, and the prevention and treatment of some diseases such as cancer (9).

1.1.3. Host-microbe interactions: parasitism

Parasitism refers to an organism, which benefits from the interaction with a host while also causing it harm. Bacterial species, which are parasitic, are considered to be pathogenic and are the cause of a variety of diseases in a host. Most pathogenic bacteria produce virulence factors, which are necessary to establish infections, produce diseases and enable their survival within the host

environment. Virulence factors include bacterial toxins, cell surface proteins that mediate bacterial attachment, cell surface carbohydrates and proteins that protect a bacterium, and hydrolytic enzymes that may contribute to the pathogenicity of the bacterium (10). The host environment is therefore, a battlefield between the host immune system and the pathogenic bacteria, and an arms race exists with bacteria evolving novel strategies to attack the host, which in turn evolves counter defense mechanisms. The round of attack and counter defense between organisms results in host–pathogen co-evolution. Coevolution between the nematode *Caenorhabditis elegans* and its natural pathogen *Bacillus thuringiensis* is one of the examples that favors and maintains pathogen virulence (11). Moreover, continuous use of antibiotic treatment against bacterial pathogenicity allows the bacteria to develop resistance and co-evolve. To this end, it is hoped that recently uncovered molecular mechanisms of host-pathogen interactions and well-characterized bacterial proteins and lipids (modulins) involved in host manipulation will contribute to new pathogen specific anti-modulin drug discovery (12).

Mutualistic, commensal and pathogenic associations are part of the continuous interactions between host-bacteria partnership and identified and distinguished by specific benefits derived by one or both members of the association. Bacterial genetic diversity, capacity for rapid growth, high population density, host immune response, physiology diet and metabolism are some of the specific conditions that can influence the switch from mutualism to commensalism or commensalism to opportunistic pathogenicity and the co-evolution of bacterial-host interactions (Figure.1) (1).
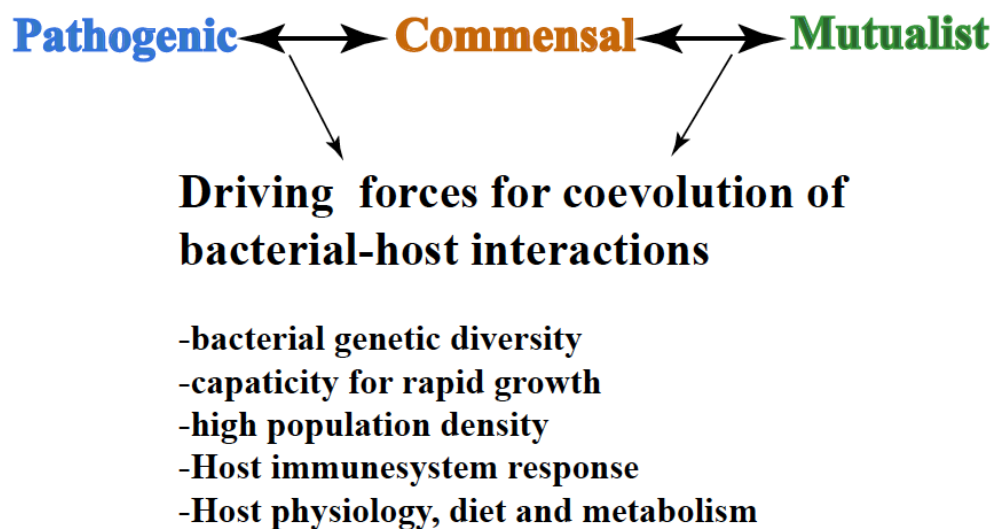

Figure 1. Driving forces for coevolution and switch of bacterial-host interactions. (Adapted from Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science. 2001;292: 1115–1118.) (1)

1.1.4. Human microbiota and challenges

All the bacteria inhabiting a human host together form the human microbiota. Microbiota provides essential functions to human development, immunity, metabolism, health and physiology and are sometimes considered the "forgotten organ". Recent studies have shown that disruption of the microbiota is associated with a broad range of diseases, including obesity, insulin resistance that is a precursor to type 2 diabetes, metabolic syndromes, autoimmune disorders, autism, neurodevelopmental diseases, inflammatory diseases, cancer and aging (13). Therefore, exploring the molecular mechanism of human-microbiota interactions will contribute our understanding of the microbiomes influence on human health, diseases and how to utilize and manipulate the microbiome to improve host health. However, it is challenging to study the microbiomes effect on human health at the system level considering i) the heterogeneity of human genomes, ii) the highly complex and heterogenic gut microbiota iii) the lack of knowledge of gut microbiota genetics and culturing methods (14). Therefore, many research groups utilize experimental models such as germ-free mice, nematodes including *Caenorhabditis elegans* and insects such as *Drosophilae melanogaster* for gut microbiota research.

1.2. Nematodes as model organisms for host-microbiome interactions

1.2.1.The nematode *Caenorhabditis elegans* as model organism

The nematode *C. elegans* was introduced in the second half of the twentieth century by Sydney Brenner as a model to study developmental and neurobiology (15). *C. elegans* is a free-living nematode, which can be grown in the lab on monoxenic bacterial cultures, most often consisting of the uracil auxotroph *Escherichia coli* mutant strain OP50, at temperatures between from 15°C to 25°C. In addition, it has a short developmental cycle of about three days under laboratory conditions. In its natural environment *C. elegans* mainly feeds on bacteria growing on rotting plant material, such as fruits, stems and flowers (16). In the presence of food and at low population density, *C. elegans* develops directly from an embryo through four larval stages to an adult. In the absence of food and at high population density, *C. elegans* may arrest its development and form dauer larvae, an alternative developmental stage that takes place following the second molting phase and is highly stress resistant and capable of surviving harsh, adverse conditions. *C. elegans* has two sexes: hermaphrodites, which are modified females that produce a limited amount of sperm and standard

males. *C. elegans* has been developed into a perfect system: it`s genome is complete (16) within estimated 50% of its genes having clear homologs in humans (17).

Additionally, *C. elegans* has a simple, well-characterized, transparent body that enables visualization and characterization of cells and phenotypes. Therefore, changes in individual gene expression throughout development and under different environmental conditions can be monitored using transgenic florescent reporters (18). Furthermore, forward genetic tools but also reverse genetic tools such as RNAi, CRISPR/ Cas9, are available for functional investigations. Finally, imaging, transcriptomics, and proteomics have been established to investigate various biological questions regarding development, neurobiology, host–pathogen interactions, and aging studies.

Given that *C. elegans* i) has a short life cycle, ii) can be handled cost efficiently, iii) can be maintained as germ-free worms, iv) provides high-throughput applicability, it represents a powerful model organism to study interactions between the host, the microbiome, and the environment. Indeed, recent studies have shown that bacteria influence *C. elegans* development, behavior, metabolism and innate immunity with bacteria produced secondary metabolites also shown to regulate *C. elegans* longevity (19).

1.2.1.1. The *C. elegans* natural microbiome

C. elegans is a cosmopolitan species found all around the world, often on rotting fruit, where they likely consume nutrient-rich substrates and bacteria. (20). By utilizing high throughput 16S sequencing and metagenome analysis, together with worldwide sampling of rotten fruits (apples, orange, cactus fruit, and black bryony) and a potential association with a vector (snail), have improved our understanding of the composition of the wild *C. elegans* microbiome. *Enterobacteriace*, *Pseudomonadaceae*, *Xanthomonadaceae* and *Sphingobacteriaceae* have been identified as most abundant phylums in the *C. elegans* gut. At the family and genus levels, the most common representatives are: in the *γ-Proteobacteria*, the families *Enterobacteriaceae* (*e.g.*, genus *Enterobacter*), *Pseudomonaceae* (*Pseudomonas*), and *Xanthomonadaceae* (*Stenotrophomonas*); in the *α-Proteobacteria*: *Acetobacteriaceae* (*Acetobacter*, *Gluconobacter*, and *Acetogluconobacter*, common in fruits); in the *Bacteroidetes*: *Flavobacteriaceae* (*Flavobacterium* and *Wautersiella*) and *Sphingobacteriaceae* (*Sphingobacterium*); in the *Firmicutes*: *Lactobacillaceae* (*Lactobacillus*), *Streptococcaceae* (*Lactococcus*), and *Leuconostocaceae* (*Leuconostoc*); and *Actinobacteria*, such as *Microbacteriaceae*, indicating the *C. elegans* microbiome has great diversity (21) (Figure 2).
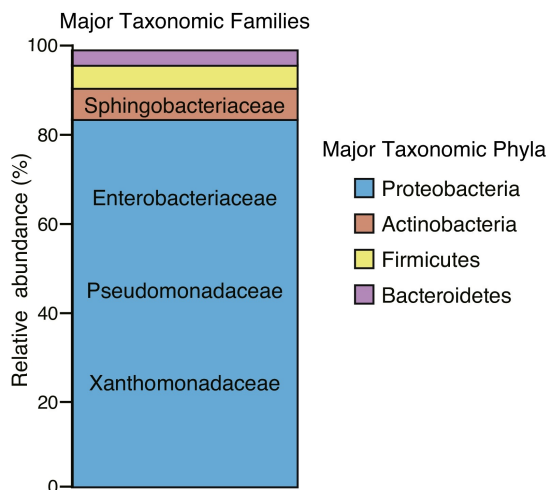
Figure 2.The structure of the native microbiome in *C. elegans*.

The most abundant phyla and families are indicated. Phyla are represented by color and families are represented by name. This figure was taken from Zhang, J., Holdorf, A. D., & Walhout, A. J. (2017). *C. elegans* and its bacterial diet as a model for systems-level understanding of host-microbiota interactions. *Current opinion in biotechnology*, *46*, 74–80. (14).

In addition to culture-independent *C. elegans* microbiome analysis, researchers isolated, cultured and identified 564 bacterial strains by 16S ribosomal gene sequencing. By assaying a combination of physiological measures, growth rates and induction of stress and immune response genes, researchers identified around 80% of these bacterial species from the natural habitats of *C. elegans*. Many bacteria support growth and healthy development, with only 20% of those natural isolates are "harmful" (impair growth, kill, activate stress/immune reporters) or "intermediate" (mixed responses). Most of the *Proteobacteria*, including *Enterobacteriaceae, Gluconobacter, Enterobacter, Providencia* and also most *Lactococcus* strains were more "beneficial" to *C. elegans*. The most deleterious genera included Bacteroidetes, such as *Chryseobacterium* and *Sphingobacterium*, and potentially pathogenic *Gammaproteobacteria* (e.g., *Xanthomonas* and *Stenotrophomonas*). Interestingly, isolates within genera varied in influence on *C. elegans* physiology (e.g., measured with the help of stress reporter genes or growth characteristics), with the exception of *Gluconobacter*, suggesting the importance of strain-level differences in gene content (21).

1.2.1.2. Interactions between *C. elegans* and pathogenic bacteria

C. elegans can be easily infected with a wide range of pathogenic microbes including some well-known human pathogens, such as Gram-negative bacteria of the genera *Burkholderia, Pseudomonas, Salmonella, Serratia*, and *Yersinia*; and the Gram-positive bacteria *Enterococcus, Staphylococcus,*

and *Streptococcus* by providing them as a food source. Usually, pathogenic bacteria colonize the *C. elegans* intestine and reduce its lifespan. For instance, the pathogenesis of the common human opportunistic pathogen *Pseudomonas aeruginosa* is medium dependent; when grown on a minimal medium, strain PA14 caused an infection-like process in the intestine of the animal, killing it over the course of several days, called "slow" killing. However, when grown on a rich medium, PA14 has been shown to kill *C. elegans* in a matter of hours, termed "fast" killing (22). *P. aeruginosa* appears to gradually target the *C. elegans* immune response by activation of signaling through the DAF-2/insulin receptor, resulting in reduced expression of a range of immune factors. Given this adaptation, there is some potential that *P. aeruginosa* may be a natural pathogen of *C. elegans*. Moreover, some of the pathogens attach to the nematode cuticle, such as *Microbacterium nematophilum,* which binds to the nematode rectum and postanal cuticle, induces swelling of the underlying hypodermal tissue and causes mild constipation (23). Furthermore, other pathogens secrete toxins that kill *C. elegans* without directly contacting with the worm. The Spore-forming bacterium *Bacillus thuringiensis* is one of the well-studied examples of this kind of infection. *B. thuringiensis* produces Cry and Cyt toxins as crystals during sporulation, and are one of the main factors causing the hosts death (24).

On the other hand, *C. elegans* developed innate immune responses to battle against pathogenic bacteria infections. First, *C. elegans* recognizes and avoids some bacteria such as some strains of *Serratia,* which produce the cyclic lipodepsipentapeptide serrawettin W2. G protein signaling pathways and the sole *C. elegans* Toll-like receptor TOL-1 are essential for this avoidance behavior (25). *C. elegans* can also recognize *Staphylococcus aureus* secreted molecules including toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxin C (SEC) through Toll/interleukin-1 receptor (TIR-1) and generation of 5-hydroxytryptamine (5-HT) (26). Second, the oxidative and xenobiotic stress response regulator transcription factor SKN-1 has been shown to be necessary for pathogen resistance to both Gram-negative *Pseudomonas aeruginosa* and Gram-positive *Enterococcus faecalis* infection via the TIR-1 protein and the p38 mitogen-activated protein kinase (MAPK) ortholog PMK-1 and its modulation of SKN-1 (27). In addition, *C. elegans* counters various small molecule toxins produced by pathogens to minimize the toxicity by chemically rearranging O-/N-glycosylation and unusual 3-O-phosphorylation of the resultant glucosides. Moreover, most of the antimicrobial peptides (AMPs) produced by *C. elegans* are caenopores, which enter the bacteria through the cytoplasmic membrane and eliminate pore-forming activity. AMPs are also required to handle normal bacterial food such as *E.coli* OP50. Absence of AMPs causes poor growth and accumulation of bacteria the in worm gut (28). Finally, the antiviral RNA interference machinery of *C. elegans* has been shown to contribute to the innate immune response in *C. elegans*.

Together, considerable amount of research has been done to understand the interaction between *C. elegans* and its pathogens, contributing to our knowledge of host-pathogens interactions. However, other mechanisms of interactions have yet to be discovered and more research is needed to confirm the complex interplay between different mechanisms of the immune response.

1.2.1.3. Bacterial diet and commensal bacteria influence in *C. elegans*

Most of the host-bacteria interaction studies have concentrated on interactions of *C. elegans* with pathogenic bacteria. Recently, however, the focus on the role and interactions of commensal bacteria in *C. elegans* is increasing. Initial experiments have been conducted using standard lab food *E. coli* OP50 to determine if the bacterial diets influence the worms despite the fact that *E. coli* OP50 does not colonize the *C. elegans gut*. *C. elegans can develop and reproduce on E. coli* indefinitely, but different strains of *E. coli* have been shown to influence worm development, physiology, lifespan, timing reproduction and fat storage (Table.1) (29).

Table 1. *E. coli* strains and the *C. elegans* response to feeding on them.

Escherichia coli strain	Strain description	Effect on *C. elegans*	Reference
OP50	*E. coli* B derivative. Standard *C. elegans* laboratory food. Uracil auxotroph.	Large lipid droplets, increased triacylglycerols relative to HB101.	30, 31
DA837	OP50 derivative. Streptomycin resistant.	Large lipid droplets, increased triacylglycerols relative to HB101.	31
HB101	*E. coli* K12/*E. coli* B hybrid. Laboratory food for growth of *C. elegans* in liquid culture.	Rapid development. Smaller lipid droplets, decreased triacylglycerols relative to OP50 or DA837.	31, 32
HT115	*E. coli* K12 derivative. RNAi host strain. RNAse III - deficient; IPTG - inducible T7 RNA polymerase.	Altered metabolism relative to OP50, including increase in aspartate, glutamate, lysine, glucose, betaine, lactate and *o* - phosphocholine, and decrease in acetate.	33
AroD	HT115 derivative. 3 - dehydroquinate dehydratase - deficient due to spontaneous mutation in *AroD*.	Lifespan extension relative to HT115.	34
GD1	*E. coli* HW272 derivative. Ubiquinone - deficient due to transposon insertion in *ubiG*.	Dose - dependent lifespan extension relative to OP50.	35

In nature, free-living *C. elegans has been identified to interact with* a wide variety of microbes as indicated above. Interestingly, *C. elegans* is able to distinguish pathogenic bacteria and beneficial bacteria based on taste and olfaction and is attracted to many of the more beneficial bacteria and are repelled by many pathogens (36). Moreover, *C. elegans* increasingly releases the neurotransmitter serotonin onto interneurons to determine whether to feed or leave the bacterial food source (37). For instance, *Bacillus mycoides* and *Bacillus soli* are preferred by *C. elegans* over the strain *E. coli* OP50. *Bacillus subtilis*, often found in the natural habitat of *C. elegans*, remains in the *C. elegans* intestine and produces fengycin, which act as antibiotic and protects *C. elegans* against *Bacillus thuringiensis* infection (38). In addition, *Bacillus megaterium* and *Pseudomonas mendocina*, which

have been isolated from *C. elegans* natural environments, induce a resistance response and protection against *P. aeruginosa* infection through the PMK-1-dependent response pathways (39). Another bacterial diet that protects against pathogenic bacteria is *Lactobacillus casei*. This bacterium protects *C. elegans* from the effects of *Klebsiella pneumonia* by triggering a toll-like receptor mediated receptor for activated C kinase 1 dependent p38 MAPK pathway to enhance *C. elegans* resistance to *K. pneumonia* infection (40).

Finally, worms obtain essential macronutrients such as carbohydrates, fats and proteins from their bacterial diet, which support the animal's growth and reproduction. Some bacteria produce essential micronutrients such as vitamins and cofactors, some of which have been recently reported to influences *C. elegans* development, metabolism and physiology and lifespan (32).

1.2.1.4. Bacterial metabolites that modulate *C. elegans* life history traits

Various studies have demonstrated bacteria-produced metabolites including nitric oxide, folate, and vitamin B12 to affect life history traits in *C. elegans*. For instance, *Bacilli* produce nitric oxide (NO), which is a crucial signaling molecule necessary for multicellular organisms. NO has been shown to increase *C. elegans* longevity and enhances stress resistance through a class of genes regulated by the HSF-1 and DAF-16 transcription factors (41). Moreover, folate (vitamin B9) has a crucial role in nucleotide biosynthesis, converting homocysteine to methionine using vitamin B12 as cofactor, and the generation of methyl donors used in various metabolic reactions such as methylation of DNA, RNA, proteins and neurotransmitters (42). When *C. elegans* is fed with *Comamonas aquatica* DA1877, it developed faster, exhibited reduced fertility and a shorter lifespan compared to animals fed on an *E. coli* OP50 diet (32). A small amount of *C. aquatica* DA1877 mixed with *E. coli* OP50 diet is sufficient to stimulate development of the worms, indicating that *C. aquatica* DA1877 provides a dilutable metabolite that influences development in *C. elegans*.

A bacterial mutant library screening with a "dietary sensor" *acdh-1::GFP* revealed that vitamin B12 is responsible for modulating the development of the worm (39). Vitamin B12 is also important for offspring development in nematodes, whereby the mother ingests vitamin B12, which is exported from the intestine into the gonad to support the development of her offspring (43,44). Vitamin B12 is exclusively synthesized by a minority of microbes and is an essential micronutrient for both humans and *C. elegans* and its biosynthesis and function will be described below (45). Taken together, these findings demonstrate the importance of bacteria as a source for micronutrients, a currently poorly explored and understood component necessary for the growth and development in *C. elegans*.

1.2.1.5. Vitamin B12

Vitamins are essential micronutrients that are commonly found as cofactors of various enzymes required for crucial biochemical reactions in all living cells. Most organisms are incapable of producing vitamins and they therefore need to be obtained exogenously. One of the most crucial vitamins for life is vitamin B12 (cobalamin), which is a cobalt containing modified tetrapyrrole. Vitamin B12 was first discovered and used against pernicious anemia by Minot and Murphy in 1926 (46). The commercial vitamin B12 (cyanocobalamin) is affiliated to the cobalamin family of compounds, which consists of a corrinoid ring and an upper and lower ligand. Adenosine, methyl or hydroxyl groups can replace the cyano group, which is the upper ligand (47). Adenosylcobalamin or methylcobalamin are the most common biological forms. Unfortunately, this essential vitamin can be only synthesized by a few bacterial species and archaea, which requires approximately 30 enzymatic steps for its complete synthesis using either aerobic or anaerobic pathway. The aerobic pathway has been best studied in *Pseudomonas denitrificans*, and the anaerobic pathway has been well described in *Salmonella typhimurium*, *Bacillus megaterium*, and *Pseudomonas shermanii* (48). Both pathways differ in terms of cobalt chelation and oxygen requirements (the aerobic pathway uses oxygen to promote ring-contraction, while the anaerobic pathway does not require oxygen in this step) (Figure 3). Several of these enzymes, which were required for vitamin B12 biosynthesis, are pathway-specific: CbiD, CbiG, and CbiK are specific to the anaerobic route of *S. typhimurium*, whereas CobE, CobF, CobG, CobN, CobS, CobT, and CobW are unique to the aerobic pathway of *P. denitrificans*.

Figure 3.Biosynthetic pathways of tetrapyrrole compounds.

Adenosylcobalamin is synthesized via the de novo or via salvage pathways. The enzymes shown in the adenosylcobalamin biosynthetic pathway originate from *P. denitrificans or S. typhimurium,* which either use the aerobic pathway or the anaerobic pathway, respectively. This figure was taken from H. Fang, J. Kang, D. Zhang, Microbial production of vitamin B12: A review and future perspectives. *Microbial Cell Factories*. **16** (2017), doi:10.1186/s12934-017-0631-y. (49).

Humans require vitamin B12 as a cofactor for two enzymes, methionine synthase and L-methylmalonyl-CoA mutase. Methionine synthase mediates the formation of methionine from homocysteine, which requires MeCbl as a cofactor. This reaction occurs in the cytosol. The second pathway takes place in the mitochondria and involves isomerization of methylmalonyl-CoA to succinyl-CoA, which is a tricarboxylic acid cycle (TCA) cycle intermediate. This reaction is catalyzed by methylmalonyl-CoA mutase and requires AdoCbl as a cofactor (50). That pathway is part of the catabolism of odd-chained fatty acids, cholesterol, and several amino acids. The excess of methylmalonyl-CoA is converted into methylmalonic acid (MMA).

Moreover, methionine can be metabolized to S- adenosylmethionine, which acts as the methyl donor in many reactions, including the methylation of DNA, histones and other proteins, neurotransmitters, and phospholipids. These methylation reactions play important roles in development, gene expression, and genomic stability. S- Adenosyl-homocysteine, the product of methylation reactions, is a potent inhibitor of many methyltransferases and is catabolized by hydrolysis to adenosine and homocysteine.

For instance, one such important methylation reaction is that of myelin basic protein. A reduction in the level of *S*-adenosylmethionine seen in pernicious anaemia (PA) and other causes of vitamin B12 deficiency produce demyelination of the peripheral nerves and the spinal column, called sub-acute combined degeneration (51). This neuropathy is one of the main presenting conditions in PA. The other principal presenting condition in PA is a megaloblastic anaemia morphologically identical to that seen in folate deficiency. Disruption of the methylation cycle should cause a lack of DNA biosynthesis and anemia.

1.2.2. *Pristionchus pacificus* as a model organism

1.2.2.1. Introducing *Pristionchus pacificus*

P. pacificus was introduced as a satellite model organism for evolutionary and developmental studies and for comparison with *C. elegans* with PS312 from Pasadena (California) established as the main laboratory strain (52). Like *C. elegans*, *P. pacificus* is a cosmopolitan nematode with various strains, which differ molecularly and developmentally from one another (53). *P. pacificus* shares many developmental and morphological features with *C. elegans*, making it a powerful model organism for comparative studies. *P. pacificus* belongs to the *Diplogastridae* family of nematodes and the last common ancestor of *P. pacificus* and *C. elegans* existed around 100 million years ago (54,55,56). Moreover, *P. pacificus* exhibits several important advantages as a model organism such as

hermaphroditism, a short generation time, and easy cultivation using again *E. coli* OP50 as a standardized food source (57). Over time many genetic, biochemical, and genomic tools have been developed in *P. pacificus*, such as forward genetics (58), a genetic linkage map (52), whole genome sequencing (59), transgenesis (60), reverse genetics (61,62), in situ hybridization (61), transcriptomics and proteomics techniques (63), which have immensely contributed to our existing knowledge of this nematode. Gene annotation following whole genome sequencing revealed many distinct features including the presence of a huge fraction of recently evolved novel genes (64). These tools have enabled the molecular and genetic characterization of several traits of ecological and evolutionary significance in *P. pacificus* (65). Altogether, these features make *P. pacificus* a good model organism for ecological, developmental, evolutionary and behavioral studies. Importantly, however, *P. pacificus* shows distinct ecological, morphological and behavioral phenotypes from *C.elegans*

1.2.2.2. *Pristionchus pacificus* ecology and development

Nematodes belonging to the *Diplogastridae* family undergo an embryonic molt from J1 to J2 before hatching, unlike in *C. elegans,* before continuing their developmental cycle. Like *C. elegans,* when *P. pacificus* is exposed to harsh conditions, such as food shortage, high temperature or high population density, they form arrested dauer larvae (66). These dauer larvae are non-feeding, with a closed mouth and a thick cuticle, which allows them to endure many environmental stresses. The dauer larvae also constitute a dispersal stage because it can attach to other invertebrates, which carry them to new habitats. Most *Pristionchus* nematodes have a necromenic relationship with scarab beetles with the dauer larvae found on living beetles (67). Here, they stay associated until the natural death of their host beetle, after which, microbes start growing on the carcass which provides favorable conditions for dauers to resume development (68) (figure 4).
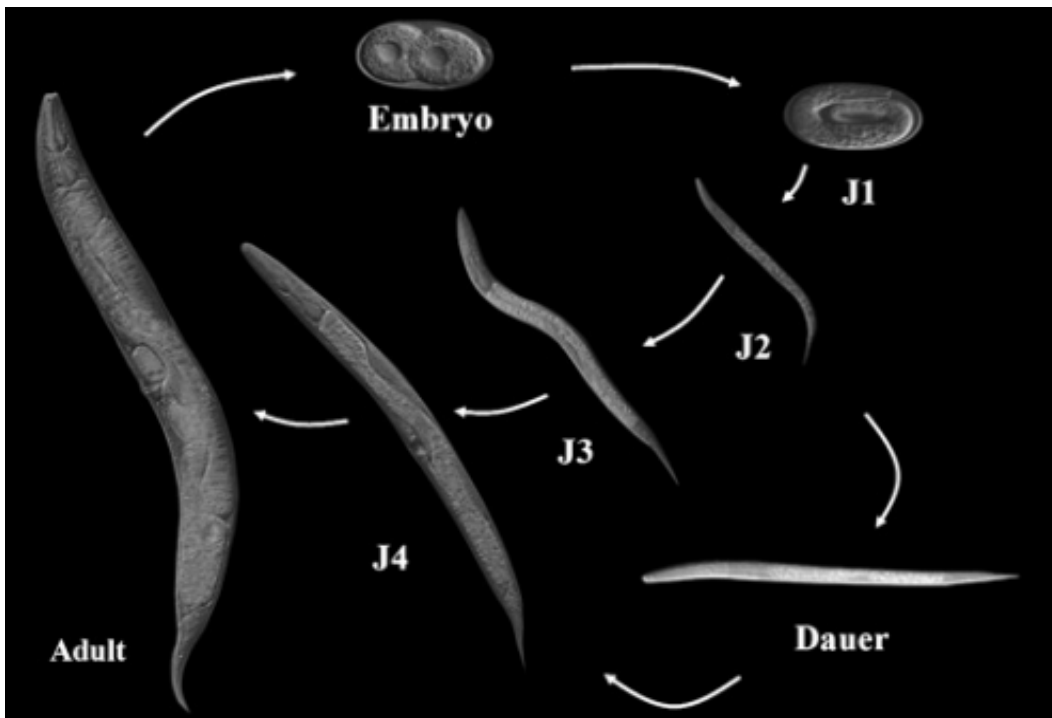
Figure 4. Life cycle of *P. pacificus*

P. pacificus has a simple life cycle that can be completed in 4 days under laboratory conditions at 20° if sufficient bacterial food is provided. Self-fertilizing hermaphrodites lay eggs, which develops into adults through larval stages (J2-J4). This figure is from R. J. Sommer, in *Nematology Monographs and Perspectives* (Entomological Society of Canada, 2015), vol. 11, pp. 19–41. (69).

P. pacificus harboring beetles have been sampled from Asia, North America, South Africa and Europe. Interestingly, *P. pacificus* has been found to be high abundant on various beetle hosts on La Reunion Island in the Indian Ocean (68,70,71). La Reunion is a young Island with a complex topographical and ecological nature, which provides an ideal setting for investigating the impacts of environment, colonization and landscape on natural nematode populations. Many *P. pacificus* isolates from this Island have contributed to our knowledge on the influence of natural variation, and changing environments to the evolutionary process (58).

1.2.2.3. Mouth form plasticity in *P. pacificus*

P. pacificus exhibits an exciting example of phenotypic plasticity. Specifically the environment influences the formation of one of two alternate mouth forms, which differ in the shape and complexity of teeth (72). Moveable teeth are a developmental and morphological novelty, specific to the *Diplogastridae* family (73,74). In early development stages an irreversible decision is made whether to develop the "eurystomatous" (Eu) or the "stenostomatous" (St) mouth form. The Eu mouth form has a wide mouth with two teeth, which allow it to feed on other nematodes in addition

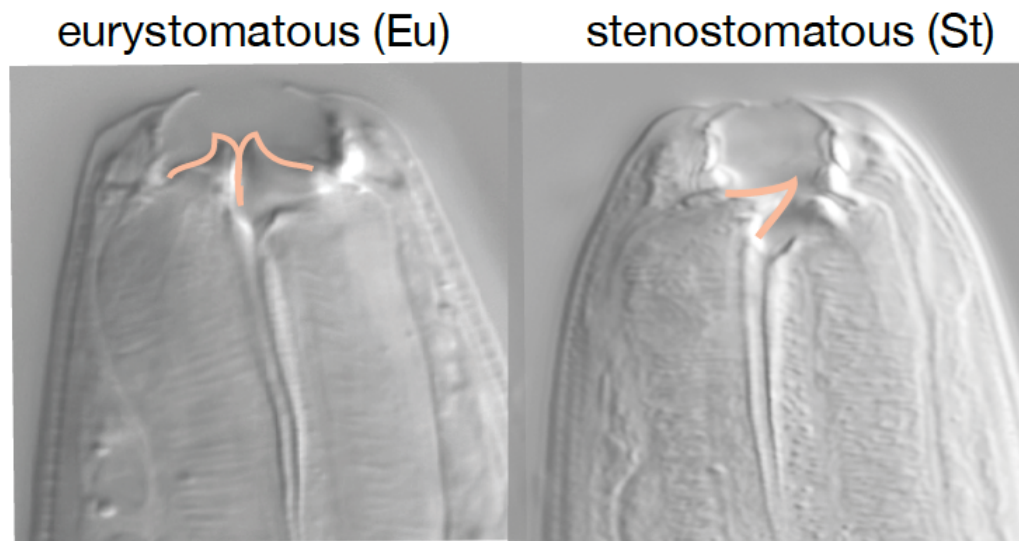to microbes. However, St animals exhibit a narrow mouth with one tooth and solely feed on microbes (Figure 5).

Figure 5. The mouth dimorphism of *P. pacificus*.

Nomarski images of *P. pacificus* "eurystomatous" (Eu) or "stenostomatous" (St) mouth form hermaphrodites. This figure is from Akduman et al., 2019 unpublished data.

Eu and St animals of *P. pacificus* have the same genetic background because the species is hermaphroditic. Under monoxenic growth conditions in the laboratory using *E. coli* OP50 as food source on NGM-agar plates, around 90% of the reference *P. pacificus* strain PS312 develop into the Eu morph (75). Therefore, both mouth forms are present at the same time in a population. The mouth dimorphism is not due to genetic variability instead a product of environmental influence, which makes it a perfect study system for phenotypic plasticity. Environmental conditions such as deprivation of bacterial food and crowding were demonstrated to promote the formation of Eu animals in a population (72). Metabolic studies have shown that the pheromone dasc#1, which has been extracted from dauer conditioned cultures, also induced Eu form suggesting a response to competition for a depleting food source (76). Mutant screens have also been conducted and several genes in the mouth-form regulatory pathway have been identified (75,77,78). The sulfatase *eud-1* (*eu*rystomatous *d*efective) was the first gene discovered in this pathway, which is a dosage-dependent "switch" gene encoding a sulfatase. *eud-1* mutants are 100% St, while overexpression of a *eud-1* transgene confers 100% Eu. Moreover, the nuclear-hormone-receptor *Ppa-nhr-40* was identified as a suppressor of *eud-1*, and regulates downstream genes (77). Two additional genes, which share the same locus as *eud-1*, *nag-1* and its paralog *nag-2* encode α-N-acetylgalactosaminidases, also additively promote the St morph (78). *C. elegans* homologs of the

epigenetic enzymes acetyltransferase *lsy-12* and methyl-binding protein *mbd-2* have also been shown to modulate mouth-form plasticity, and are attractive factors for channeling environmental cues to changes in gene regulation. Both mutants led to a global loss of activating-histone modifications, and decreased expression of *eud-1* (79). Together, these recent studies on the *P. pacificus* mouth form dimorphism have explored this phenomenon at several levels of biological organization, including morphology, feeding ecology, adaptive value, evolution, regulation by pheromones, hormones and a developmental switch mechanism.

1.2.2.4. Predatory feeding behavior in *P. pacificus*

Nematodes exhibit a vast spectrum of mouth adaptations that coincide with a great diversity in feeding behaviors and diets. (73,80). In *C. elegans*, the buccal cavity is composed of a simple tube with a more complex apparatus found in the terminal bulb where hardened discs of collagen form a grinder to aid in bacterial lysis (81). In contrast, *Pristionchus* and its relatives from the *Diplogastridae* family are capable of both microbial feeding and predatory feeding on other nematodes, based on the moveable teeth for opening the cuticle of their prey as described above. The St form appears to be optimized for bacterial food sources, whereas the Eu form optimized is for predation (79). This makes *Pristionchus* is an ideal model organism to study behavior under both morphological and ecological contexts. An initial analysis of *Pristionchus* predatory behavior revealed that tooth movement rates significantly increased during predation and becomes coupled to pharyngeal pumping in a 1:1 ratio (82). Moreover, pharmacological experiments using neurotransmitters such as serotonin, dopamine, octopamine and tyramine revealed that only serotonin triggers a predatory-like pumping and tooth movement response similar to those observed while feeding on prey (figure 6) (83).
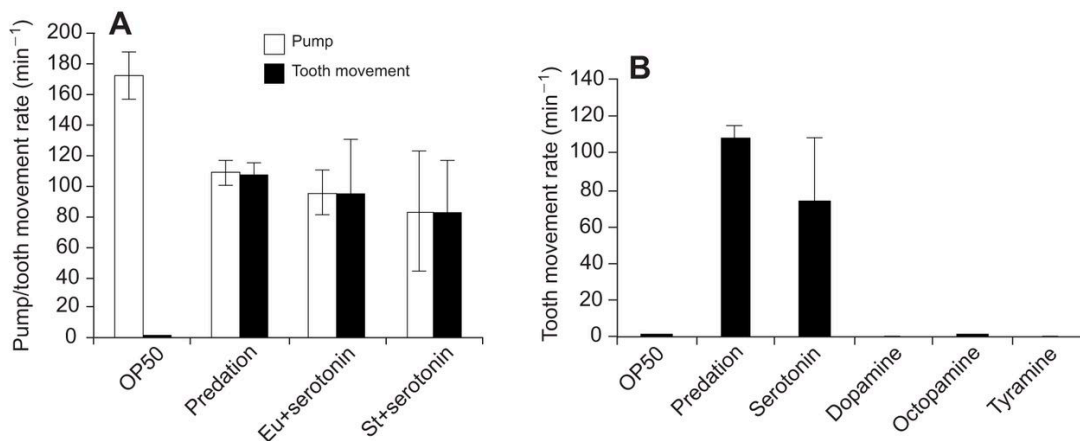
Figure 6. Predatory feeding mode is regulated by the neurotransmitter serotonin. (A) Quantification of pharyngeal pumping and tooth movement rates reveals significant differences between bacterial and predatory feeding. Pumping rate is substantially lower during predatory feeding compared with bacterial feeding, while tooth movement rate increases dramatically. The neurotransmitter serotonin (10 mmol l^{-1}) triggers a predatory-like pumping and tooth movement response in both Eu (wild-type) and St (*eud-1* mutant) animals. OP50, *Escherichia coli* OP50. Error bars represent s.d. (B) Neurotransmitter effect on tooth movement rate. Treatment with 10 mmol l^{-1} serotonin triggers a predatory-like tooth movement response. The neurotransmitters dopamine (10 mmol l^{-1}), octopamine (10 mmol l^{-1}) and tyramine (10 mmol l^{-1}) do not affect tooth movement. Error bars represent s.d. This figure is from M. Wilecki, J. W. Lightfoot, V. Susoy, R. J. Sommer, Predatory feeding behaviour in Pristionchus nematodes is dependent on phenotypic plasticity and induced by serotonin. *Journal of Experimental Biology*. **218**, 1306–1313 (2015). (83).

Serotonin is synthesized from tryptophan by two conserved enzymes the tryptophan hydroxylase (TPH-1) and 5HTP/L-dopa decarboxylase (BAS-1). In *C. elegans tph-1* mutants have a decreased pumping rate during bacterial feeding (84), and serotonin stimulates pharyngeal pumping and isthmus peristalsis mimicking the effect of bacterial food (85). In *P. pacificus*, *Ppa-tph-1* and *Ppa-bas-1* mutants decrease predation efficiency with a loss of coordination between tooth movement and pumping while predating on *C.elegans* larvae in comparison to wild-type animals. This indicates that serotonin controls the temporal coordination of pharyngeal pumping and tooth movement during predation. Moreover, serotonin also regulates other functions in *P. pacificus,* such as egg laying and stimulates the pumping rate during bacterial feeding (82).

1.2.2.5 Surplus killing behavior

Predation has long been implicated as a major selective force in the evolution of several morphological and behavioral characteristics of animals. However, another common predatory feeding behavior exhibited by predators is surplus killing, in which predators kill more prey than

they can immediately consume and then they either cache or abandon the remainder. In the initial analysis of predation in *P. pacificus* it was found that these nematodes indeed exhibit surplus-killing behavior (83). This behavior has also been referred to as excessive killing and henhouse syndrome, which was described by Dutch biologist Hans Kruuk after studying, spotted hyenas in Africa (86). Surplus killing behavior is very bewildering as the predator expends energy and there is high risk of injury for little gain. This behavior may be the product of a context-specific, adaptive foraging strategy restricted spatially and / or temporally to conditions of prey abundance. Alternatively, surplus killing may represent a context-general syndrome of high aggression that results in killing prey that is not consumed. Many predators, including large mammals such as wolves, bears and orcas, but also in birds, crayfish, spiders, and insects frequently show this behavior (87,88,89,90,91,92,93,94).

Predators mostly show surplus killing behavior i) when plenty of prey are present ii) they consume nutritious part of the prey and discard the reaming parts and iii) predating to save food for a later time or iv) to provide food for members of a pack. For instance, brown bears (*Ursus arctos*) often exclusively feed on lipid-rich tissues of Pacific salmon (*Oncorhynchus spp.*), and discard remains when numerous salmon present late in the salmon run. Moreover, bears also have been observed to kill and abandon salmon without consuming any tissue (95). Furthermore, Zimmermann et al reported that adult wolves (*Canis lupus*) are responsible for killing prey for the wolf pack, which consists pups and old wolves. Adult wolves belonging to small packs were observed to exhibit elevated surplus killing behavior in comparison to those belongs to large packs, suggesting surplus killing by small packs follow an optimal foraging strategy with only the most nutritious parts of prey being consumed to avoid the risk of encountering with humans (87). However, density dependence and food restriction alone are poor predictors of killing behavior, and the full impact of diet on surplus killing and predation in general is currently poorly understood. This PhD thesis describes a new insight into surplus killing as observed in nematodes.

1.2.2.6 *P. pacificus* self-recognition

Interestingly, *Pristionchus* species are able to recognize their own species and avoid killing self-progeny. Therefore, a self-recognition mechanism in *Pristionchus* species prevents cannibalism and facilitates the selective predation on unrelated nematode species. Self- recognition, the capacity to discriminate between self and foreign tissue, or kin has been observed and well-studied in various biological content throught all domains of life. However, predatory nematodes of the genus *Pristionchus* are (96), the first of its kind to demonstrate self-recognition behavior among nematodes. The self-recognition mechanism acts through the nematode surface and is dependent on a small

peptide called SELF-1. *self-1* is composed of an invariant domain and a hypervariable C terminus, which appears to be essential for self-recognition due to its sequence variability at the strain level (96). Hence, nematodes provide a powerful system to discover the complex genetic mechanisms behind self-recognition.

1.2.2.7. *P. pacificus* interactions with bacteria

As discussed above the free-living soil nematodes *C. elegans* and *P. pacificus* can use bacteria as their main food source. Although both nematodes are saprobic, the morphology of their pharynx differs from each other. *C. elegans* has a grinder in the terminal bulb of the pharynx that crushes bacterial cells, while *P. pacificus* does not have a grinder (Figure 7), probably a secondary loss after gaining teeth-like denticules and affecting the physiology and the innate immunity of *P. pacificus*.

Figure 7. Pharynx morphology of *C.elegans* and *P. pacificus*

(A) *C. elegans* pharynx with grinder and long, narrow mouth-like pump. (B) *Escherichia coli* OP50 crushed with the *C. elegans* grinder. (C) *P. pacificus* pharynx with no grinder and shorter, broader mouthparts. (D) *E. coli* OP50 is not completely disrupted after passage through the pharynx of *P. pacificus*. Figure from (97).

First experiments on *Pristionchus*-bacteria interactions focused on i) understanding bacteria interactions with *Pristionchus* species developing on the decaying beetle host; ii) determining the effects of the natural bacteria on nematodes; and iii) comparing *P. pacificus* and *C. elegans* immune responses to pathogenic bacteria. Metagenome analysis of unculturable bacteria from *P. iheritieri* and *P. entomophagus* showed at least 40 bacterial species are present in the nematode gut. Most of these bacterial species are animal and plant pathogenic bacteria including *Bordella sp., Burkholderia sp., Agrobacterium sp.,* and *Microbacterium sp.* (97). Moreover, some species including *Bacillus* and *Pseudomonas* and the pathogenic bacteria *Serratia* were isolated and cultured from these nematode species. Chemotaxis assays were also performed to assess the nematodes' food preference and

reaction to natural bacteria isolates. This studies revealed that *P. pacificus* is repelled by *Serratia marcescens* possibly because of its pathogenicity. Also, *P. pacificus* avoids *Bacillus thuringiensis* and insect pathogenic bacteria. Moreover, survival assays showed that it is resistant to the human pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*, unlike *C. elegans*. Resistance to some pathogenic bacteria may be explained by the absence of the grinder in the pharynx where bacteria cells could pass through alive without disruption. Furthermore, the *P. pacificus* genome harbors many more genes, which are involved in detoxification of xenobiotic compounds (98).

On the other hand, pure Cry5B toxin, isolated from *Bacillus thuringiensis*, can kill *P. pacificus,* whereas nematodes are unaffected when fed *E. coli* cells that express the same toxin (99). This observations support the idea that the absence of cracking bacteria by the grinder, because it no longer exists in *P. pacificus*, is involved in generating the observed resistance of *P. pacificus* against many pathogens. Forward genetic approaches were used to identify *P. pacificus* uncoordinated (unc) mutants that are hypersusceptible to Gram-positive pathogens. Unc mutants often possess severe muscle defects with prolonged defecation cycles resulting in an extended time of bacterial residence in the intestine. This prolonged exposure to pathogens increases fatality (100). Hence, intact peristalsis protects intestine from pathogenic bacteria colonization.

Furthermore, germline ablation in *C. elegans* prolonged life span and elevated resistance to pathogens (101). This same phenomenon was also observed in *P. pacificus* worms, which also showed increased longevity and resistance to pathogenic bacteria when worms underwent gonad ablation (102). This mechanism acts through diverse downstream genes that modulate many processes, such as translation initiation, proteasome maintenance, insulin signaling and lipid metabolism, and the DAF-16/FOXO transcription factor. These findings revealed that the same pathways regulate longevity and innate immunity, and the influence of the reproductive system on lifespan and innate immunity is conserved in nematodes. While previous studies provided important insights into *P. pacificus* defense mechanisms against infections, how *P. pacificus* interacts with commensal nonpathogenic bacteria and how these bacteria influences nematode development, behavior and morphology is currently well understood.

2. The aim of the thesis

My goal was to elucidate the influence of natural bacteria isolates on development, behavior and morphology of the nematode *P. pacificus*. *P. pacificus* has been cultured for countless generations in the lab on the *E.coli* OP50 strain, which does not interact with *P. pacificus* in nature. However, current studies on the microbiome revealed undeniable impact of microbiota on host traits. Recent studies from our lab focused on *P. pacificus*-pathogenic bacteria interactions, whereas, little is known on how commensal bacteria affect the *P. pacificus*. Therefore, I wanted to study *P. pacificus* - commensal bacteria interactions, with an emphasis on how commensal bacteria modulate predatory feeding behavior in *P. pacificus*.

To study the interactions between *P. pacificus* and its natural bacteria, I have carried out a culture-based approach to isolate and investigate nematode-associated bacteria from beetle hosts, figs, and soil. I then identified bacterial isolates as pathogen or commensal by performing *P. pacificus* survival and chemotaxis assays. My data shows that most of the isolated bacteria from natural habitats are commensal to *P. pacificus* worms, and they seem to be more attractive to them than the standard *E. coli* OP50 strain. Then, I focused specifically on commensal bacteria isolates and investigated a potential influence on *P. pacificus* predatory behavior. *Novosphingobium sp.* showed the highest influence on predatory behavior, and I subsequently found that vitamin B12-producing microbiota modulate development and surplus killing behavior in *P. pacificus*, and that vitamin B12's influence on development is conserved across several nematode species.

3. Results

3.1. Culture-based analysis of *Pristionchus*-associated microbiota from beetles and figs for studying nematode-bacterial interactions

Nermin Akduman, Christian Rödelsperger, Ralf J. Sommer

PLoS ONE. 13 (2018), doi:10.1371/journal.pone.0198018.(103)

3.1.1 Contribution table

| Author	Author position	Scientific ideas %	Data generation %	Analysis & Interpretation %	Paper writing %
Nermin Akduman	First	75	100	60	50
Dr. Christian Rödelsperger	Second	25	0	40	50
Title of paper:		Culture-based analysis of *Pristionchus*-associated microbiota from beetles and figs for studying nematode-bacterial interactions			
Status in publication process:		Published in 2018			

Culture-based analysis of *Pristionchus*-associated microbiota from beetles and figs for studying nematode-bacterial interactions

Nermin Akduman, Christian Rödelsperger, Ralf J. Sommer*

Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Max-Planck-Ring 5, Tübingen, Germany

* ralf.sommer@tuebingen.mpg.de
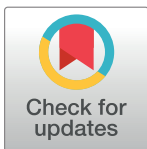
Abstract

The interplay with bacteria is of crucial importance for the interaction of multicellular organisms with their environments. Studying the associations between the nematode model organisms *Caenorhabditis elegans* and *Pristionchus pacificus* with bacteria constitutes a powerful system to investigate these interactions at a mechanistic level. *P. pacificus* is found in association with scarab beetles in nature and recent studies revealed the succession and dynamics of this nematode and its microbiome during the decomposition of one particular host species, the rhinoceros beetle *Oryctes borbonicus* on La Réunion Island. However, these studies were performed using culture-free methods, with no attempt made to establish bacterial cultures from the beetle-nematode ecosystem and to investigate the effects of these microbes on life history traits in *P. pacificus*. Here, we establish and characterize a collection of 136 bacterial strains that have been isolated from scarab beetles and figs, another *Pristionchus*-associated environment, as a resource for studying their effect on various nematode traits. Classification based on 16S sequencing identified members of four bacterial phyla with the class of Gammaproteobacteria representing the majority with 81 strains. Assessing the survival of *P. pacificus* on individual bacteria allowed us to propose candidate groups of pathogens such as Bacillaceae, Actinobacteria, and *Serratia*. In combination with chemoattraction data, it was revealed that *P. pacificus* is able to recognize and avoid certain groups of pathogens, but not others. Our collection of bacterial strains forms a natural resource to study the effects of bacterial diet on development and other traits. Furthermore, these results will form the basis of future studies to elucidate the molecular mechanisms of recognition and pathogenicity.

Introduction

Bacteria form an integral part of the ecology of all living beings and the influence of the gut microbiota on human health has been increasingly recognized during the last decade [1]. Nematodes like *C. elegans* are an excellent model to study the interactions between bacteria and their hosts [2], because they are easy to grow using monoxenic bacterial cultures, eg.

Escherichia coli OP50 as food source. In addition, worms as well as bacteria are genetically tractable, which can provide detailed mechanistic insights into the interaction between host and bacteria and their impact on development and behavior [3,4]. We study the nematode *Pristionchus pacificus* a close relative of the rhabditid *C. elegans*, but belonging to the Family Diplogastridae [5]. *P. pacificus* and *C. elegans* have been estimated to have diverged 280–430 million years ago [6]. *P. pacificus* is found in a necromenic association with scarab beetles [7], i.e. nematodes are maintained as growth-arrested dauer larvae on the beetle and upon the beetle's death resume development and reproduce. They feed on the microorganisms growing on the beetle's carcass and recent decomposition studies using the rhinoceros beetle *Oryctes borbonicus* from La Réunion Island as host have indicated that the decaying beetles and *P. pacificus* have largely overlapping microbiomes [7]. While *P. pacificus* and *C. elegans* share many biological features, such as the mode of reproduction, the presence of an alternative developmentally arrested dauer stage, and the same chromosome number, nematodes of the *P. pacificus* lineage have gained the ability to form tooth-like structures that allow them to predate on other nematodes [8–11]. Interestingly, these feeding structures represent an example of phenotypic plasticity because *P. pacificus* can form two alternative mouth forms with stenostomatous animals being strict bacterial feeders, whereas eurystomatous animals are omnivorous feeders that can also kill other nematodes [11]. Whether or not these predatory structures are formed during development is environmentally controlled. Thus, *Pristionchus* mouth-form plasticity represents a developmental decision similar to other examples of phenotypic plasticity in animals, such as the caste system in social insects [12] or color patterns in butterfly wings [13]. To explore the full range of environmental variables that potentially influence developmental decisions, we have recently started to modify culture conditions [14] and tested food sources other than *E. coli* OP50 bacteria [15]. Specifically, these studies have shown that growth of worms on yeasts or in liquid culture conditions has an effect on mouth-form plasticity [14,15]. The association of *Pristionchus* nematodes with scarab beetles is stable over millions of years of evolution and has resulted in more than 30 *Pristionchus* species that are found worldwide, often in species-specific interactions with scarab beetles [16]. In addition, a recent study discovered a second branch of the *Pristionchus* genus that is found in association with figs and fig wasps [17]. Strikingly, fig-associated *Pristionchus* species are even capable of producing up to five different mouth morphotypes, whereas beetle associated *Pristionchus* are usually dimorphic [17]. Evidence supported that distinct morphotypes were associated with the degree of maturity of figs and it was hypothesized that the presence of certain bacteria may trigger these developmental decisions. Unfortunately, we failed to cultivate fig-associated *Pristionchus* nematodes permanently under laboratory conditions, which prohibited the further elucidation of environmental cues controlling the development of individual morphs.

The interactions between *Pristionchus* nematodes and bacteria have been studied in the last decade largely by exploring beetle-derived bacteria from Germany and other European sampling sites [18]. These original studies had indicated differences in the response of *P. pacificus* and *C. elegans* to *Bacillus* ssp., which have initiated large-scale studies of Bacilli and their effect on both nematodes [19–22]. However, *P. pacificus* does not normally occur in Europe and the original bacterial isolates mentioned above were obtained from *P. maupasi* and *P. entomophagus*.

To further study the interactions between *P. pacificus* with its natural bacteria, we have carried out a culture-based approach to isolate and investigate nematode associated bacteria from three different locations in Asia, Africa and the Indian Ocean and used both hosts, beetles and figs. Specifically, we isolated bacteria from figs retrieved from Vietnam, South Africa, and La Réunion Island and from scarab beetles from La Réunion Island, which forms a hotspot of *P. pacificus* diversity [7,23]. In total, we classified 136 bacterial isolates based on their 16S

ribosomal RNA sequences. We test the nematodes´ capability to survive on these bacteria and their chemoattractive potential relative to standard *E. coli* OP50 cultures. Our data show that most of the isolated bacteria support growth of *P. pacificus* worms and most bacteria seem to be more attractive than the standard *E. coli* OP50 strain. Furthermore, the finding of a weak correlation between survival and chemotaxis data raises the question to what extent nematodes can sense which food sources are suitable for them.

Materials and methods

Nematode and bacterial culture conditions

The wild type strain of *P. pacificus* (PS312) was grown at 20˚C on nematode growth medium (NGM) seeded with *E. coli* OP50 before use in experiments. Bacterial strains were cultured on following growth media: LB agar (1% tryptone, 0.5% yeast extract, 1% NaCl and 1.5% agar), NGM [24], YPD agar (2% bacteriological peptone, 1% Yeast extract, 2% Glucose and 1.5% Agar), NA (Thermo scientific, Oxoid, CM0003), TSA (1.5% tryptone, 0.5% soytone –enzymatic digest of soybean meal, 0.5% sodium chloride, 1.5% agar), PDA (Difco™ Potato Dextrose Media, BD, 1.5% Agar).

Sample collection and isolation of wild bacteria

We collected different beetle species (*Oryctes borbonicus*, *Adoretus* sp., *Hoplia sp*. and *Amneidus* godefroyi) from La Réunion Island (Fig 1A) using sweeping nets, black light traps and pitfall traps baited with dung [25]. Only adult beetles were collected before being transferred to the laboratory alive. To avoid contamination by human associated bacteria, all sample collections were done wearing gloves. Under sterile conditions animals were sacrificed by cutting them in half transversely and all body parts were placed on LB agar plates. Bacteria were only isolated from beetles that also showed the presence of *Pristionchus* nematodes. Plates that were negative for *Pristionchus* but that were positive for other nematodes were discarded. Isolated bacteria were spotted on LB plates and colonies were singled out for two rounds to get pure bacterial strains. For genotyping, bacterial strains were sub-cultured and then prepared for sequencing using PCR amplification of 16S ribosomal RNA genes. Permits for beetle samplings on La Réunion Island were provided by Sylvain Leonard from the Office National des Forets and Benoit Lequette from the Parc National de La Réunion between 2012 and 2017. Note that the research permits did not allow the disclosure of the exact sampling localities because several beetle species are endangered (i.e. *Oryctes borbonicus*).

To isolate bacteria from figs, we collected several fig species including *F. mauritiana* (La Réunion), *F. sycomorus* (South Africa), and *F. racemosa* (Vietnam) (Fig 1B and 1C). Individual figs were dissected under sterile conditions and the presence of *Pristionchus* nematodes was confirmed. 500 μl of fig juice was extracted with a sterile pipette and suspended in sterile PBS, and aliquots were spread on LB, NGM, YPD, NA, TSA, and PDA agar plates and then grown for 1–2 d at 30˚C. Single colonies were isolated from plates, grown in LB (shaking at 180 rpm, 30˚C) or until significant growth was achieved, and frozen at −80˚C in 25% glycerol stocks. Permits for fig samplings on La Réunion Island were provided by Benoit Lequette from the Parc National de La Réunion between 2014 and 2016.

Bacteria identification

Each bacterial colony was grown overnight in LB broth and DNA was extracted using Epicenter MasterPure DNA purification kit (Illumina, San Diego, USA). Polymerase chain reaction (PCR) amplification of bacterial 16S rRNA genes was carried out in 25 μl reactions using a
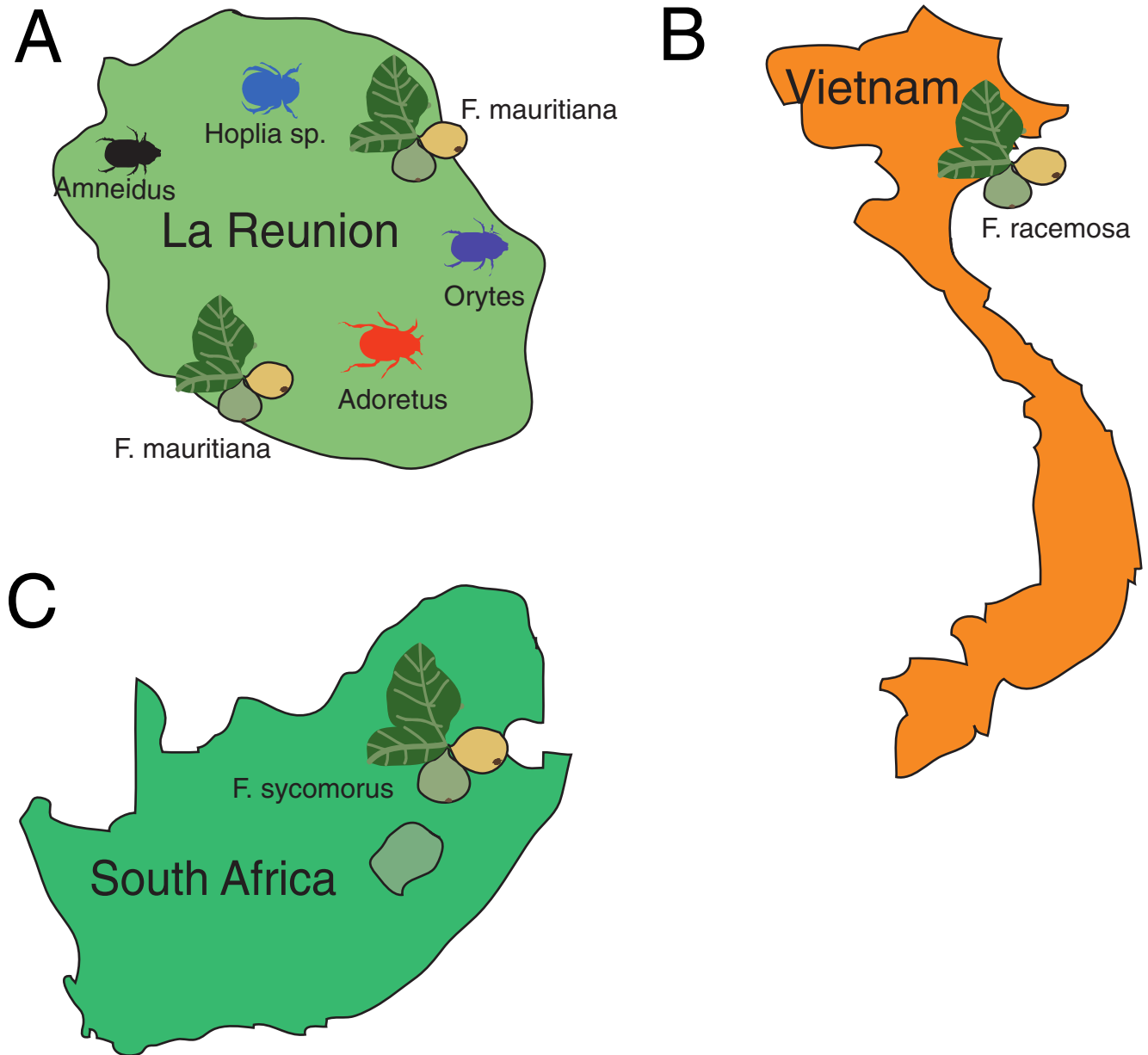
Fig 1. Regional maps of beetle and fig sampling sites. (A) Map of La Réunion Island showing the approximate beetle and fig sampling sites. *O. borbonicus*, *Adoretus* sp., *Hoplia sp.* and *A. godefroyi* beetles were collected to isolate *Pristionchus*-associated bacteria. Similarly, *F. mauritiana* figs were sampled and processed for bacteria isolation. (B) and (C) *F. sycomorus* (Brummeria, Pretoria, South Africa) and *F. racemosa* (Hanoi, Vietnam) figs were dissected under sterile conditions from *Pristionchus*-positive specimens to isolate bacteria.

https://doi.org/10.1371/journal.pone.0198018.g001

universal primer set SSU 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and SSU 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) [18]. Thermal cycling conditions were as follows: 3 min at 95˚C followed by 30 cycles of 15 s at 95˚C, 30 s at 55˚C, 1.5 min at 72˚C, and a final step of 8 min at 72˚C. A typical reaction contained 2 μl 10x PCR buffer, 2 μl 2·mmol·l^{-1} dNTPs, 1μl 10 μmol·l^{-1} 27f, 1μL10 μmol·l^{-1} 1492r, one unit of Taq DNA polymerase, 12.8·μl H$_2$O and 1μL of bacterial DNA. PCR amplicons were visualized by standard agarose gel electrophoresis [26]. All high quality 16S rRNA gene sequences of bacteria were classified by the SILVAngs webservice of the SILVA database [27].

Survival assays

Bacterial liquid cultures were established by inoculating 5ml LB with a single bacterial colony. Subsequently, cultures were grown overnight at 30˚C. Bacterial suspensions (50μl) were spread with an L-shaped spreader on NGM medium petri dishes with diameter of 6cm and were incubated overnight. Twenty young adult *P. pacificus* worms that were well fed on *E. coli* OP50, were washed five times with PBS and picked to intermediate plates seeded with test bacteria to reduce contamination, a standard procedure in nematode survival assays. One hour later, worms were picked to the final assay plates seeded with test bacteria. Each plate was kept at 20˚C. Survival of worms was monitored daily for 5 days. Nematodes were transferred every two days to fresh plates to prevent misidentification of original worms from offspring. Mortality was determined by prodding worms with a metal pick and nematodes that did not respond were considered dead. In total, we performed three biological replicates per bacterial strain.

Chemotaxis assays

Chemotaxis assays were modified from previous studies [28,29]. Briefly, 20 μl of overnight bacterial suspension was placed 0.5 cm away from the edge of a 6 cm Petri dish filled with NGM medium. The same amount of *E. coli* OP50 was placed on the opposing side acting as the counter attractant. Approximately 50–200 J4/adult stage *P. pacificus* individuals were placed at the edge of the plate, equidistant to each of the bacterial spots. All nematodes were previously fed on *E. coli* OP50. Plates were incubated at room temperature. After 3h the number of nematodes found in each bacterial spot was recorded. A chemotaxis index was used to score the response of the nematodes, which consisted of: number of nematodes in the region spotted with test bacteria minus the number of nematodes in control bacteria spots, the result was divided by the total number of nematodes counted [29]. This gave a chemotaxis score ranging from –1.0 (repulsion) to 1.0 (attraction). Three plates were used per replicate, and the procedure was repeated four to six times for each bacterium.

Statistical analysis

For each bacteria analysed, we averaged the survival and chemotaxis values from all replicates and employed a Wilcoxon rank-sum test to test for significant differences between taxonomic groups. P-values were corrected for multiple testing with the Bonferroni method. Correlation between survival and chemotaxis data was calculated as Spearman correlation. All analyses and plots were done using the statistical program R.

Results

A bacterial strain collection from *Pristionchus*-associated environments

We used fig and beetle samples provided by our collaborators, to isolate and cultivate a total of 136 bacterial strains (S1 Table). Specifically, 80 different bacterial strains were isolated from beetle species (*O. borbonicus*, *Adoretus* sp., *Hoplia sp*. and *A. godefroyi* collected from La Réunion Island) (Fig 1A). In addition, we isolated 26 bacterial strains from *F. mauritiana* (La Réunion), 21 strains from *F. racemosa* (Vietnam) and nine strains from *F. sycomorus* (South Africa)(Fig 1A). Each strain was classified after sequencing a fragment of the 16S ribosomal RNA gene. Overall, we isolated strains belonging to four bacterial phyla, Proteobacteria, Firmicutes, Bacteriodetes, and Actinobacteria (Fig 2A). The same four phyla were also present when only considering bacterial isolates from beetles (Fig 2B). These findings are consistent with the recent high throughput sequencing of the microbiome of *O. borbonicus* and *P. pacificus* [7]. Out of the 104 isolated strains of Proteobacteria, 47 are representatives of the family
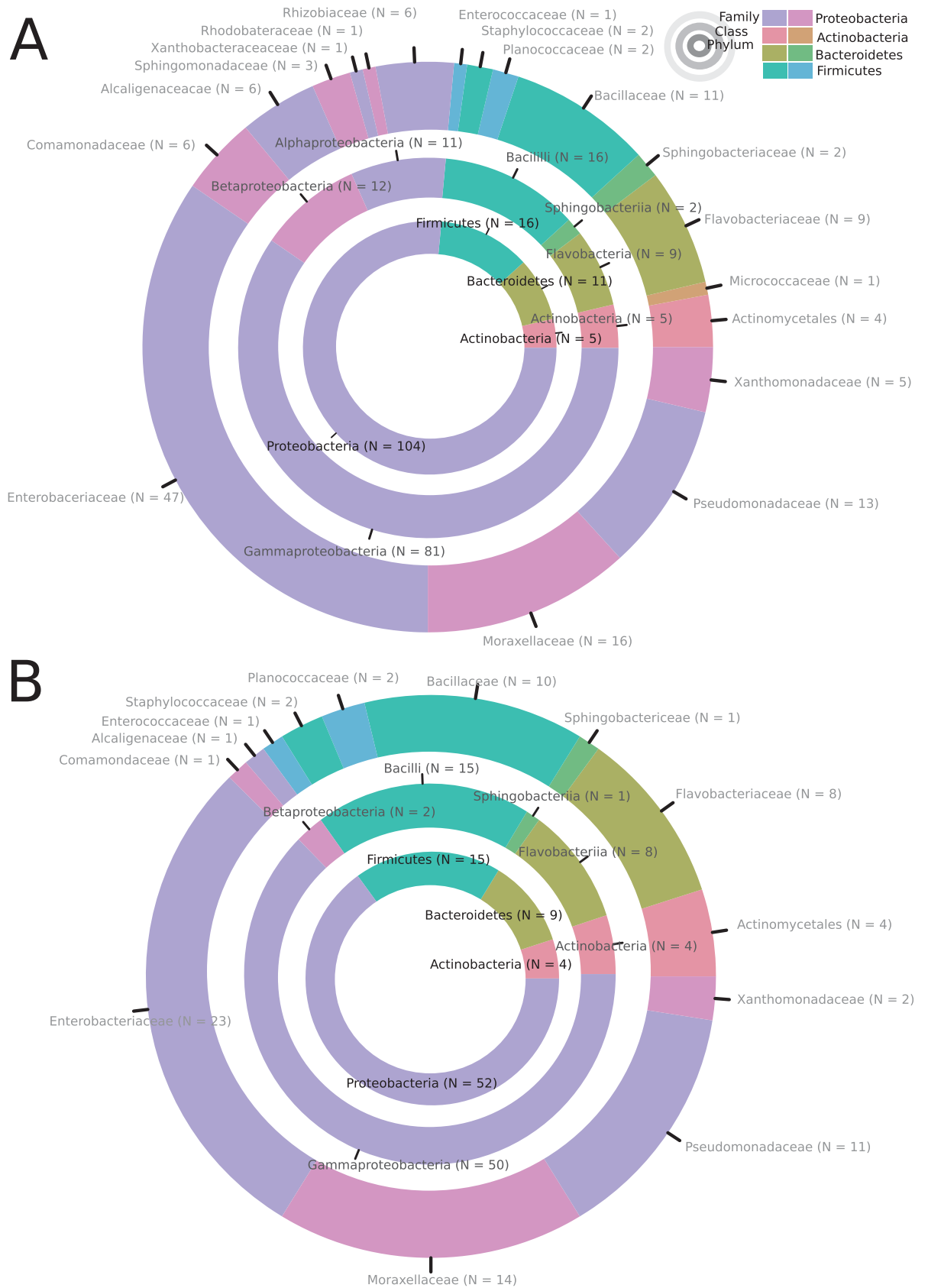
Fig 2. Taxonomic distribution of bacterial isolates. (A) Circles show the distribution of bacterial strains at the level of phyla (innermost circle), class (middle circle), and family (outermost circle) based on classification of the 16S ribosomal RNA gene [27]. Proteobacteria are by far the largest group (N = 104 strains). (B) Distribution of bacterial strains that were isolated from beetles on La Réunion Island.

Enterobaceriaceae, which has also been found to be by far the most abundant family of bacteria in decaying beetles [7]. Thus, our culture-based method is largely consistent with the culture-free results of *O. borbonicus*-associated microbes and provides a collection of 136 bacterial strains for laboratory studies.

Most bacterial isolates are not pathogenic to *P. pacificus*

Next, we tested how well *P. pacificus* strains can survive on the isolated bacteria. To this end, we exposed 20 young adults to individual bacterial strains and counted the number of surviving worms after five days. Control experiments performed on *E. coli* OP50 showed a survival rate of between 95–100%. In our experimental setup, worms could survive on most of the tested bacteria (Fig 3). Fig 3 shows the result of the survival tests at the level of bacterial classes and selected families. Survival on Bacilli strains was significantly lower (Wilcoxon Rank-Sum test, Bonferroni corrected p-value < 0.05) than on Flavobacteria and Alphaproteobacteria (Fig 3A). In contrast, sample sizes of Actinobacteria and Sphingobacteria were too low to reveal statistically significant differences in nematode survival in comparison to other bacterial classes. However, investigating the survival patterns at higher taxonomic resolution, we found that the lower survival on Bacilli strains is largely due to members of the family Bacillaceae (Fig 3B) and the high variability in Gammaproteobacteria can be attributed to variability in the family Enterobacteriaceae. At the level of genera, the variability within Enterobacteriaceae appears to be caused by lower survival on individual strains belonging to *Serratia*, *Morganella*, *Enterobacter*, *Klebsiella* and *Pectobacterium* (results were not statistically significant after multiple testing correction, Fig 3C). While some *Serratia* strains have previously been described to be pathogenic to *P. pacificus* [30], it also seems that some *Enterobacter* and the human pathogen *Klebsiella* strains can also be pathogenic to *P. pacificus*.

P. pacificus nematodes are attracted to most bacterial isolates

To test whether *P. pacificus* nematodes are attracted towards the isolated bacteria, we performed chemotaxis assays by giving worms the choice between two alternate food sources. Specifically, we used one spot of the target bacteria opposite one spot of an equal volume *E. coli* OP50 and counted the number of worms in each of the spots after three hours. Subsequently, we then calculated a chemotaxis index (CI). A CI of -1 indicates repulsion from the test bacterium, whereas a CI of 1 indicates attraction. Our results show that most bacterial isolates are preferred by *P. pacificus* as opposed to control spots. However, individual strains of the classes Actinobacteria, Flavobacteria, and Bacilli showed negative CIs (Fig 4A). Within Bacilli, the repulsive effect was mostly due to the family of Bacillaceae (Fig 4B), whereas other families of Bacilli frequently showed positive CIs indicating strong strain specificity.

Weak correlation between survival and chemotaxis

Combining the results of the survival and chemotaxis assays it appears as if *P. pacificus* can recognize and escape from certain pathogens. For example, certain strains of Bacillus and Actinobacteria show low survival of *P. pacificus* and strong repulsion in chemoattraction assays (Fig 5). In addition, most non-pathogenic strains seem to be preferred by worms over *E. coli* OP50 control spots. To test to what extent *P. pacificus* can distinguish suitable food sources from
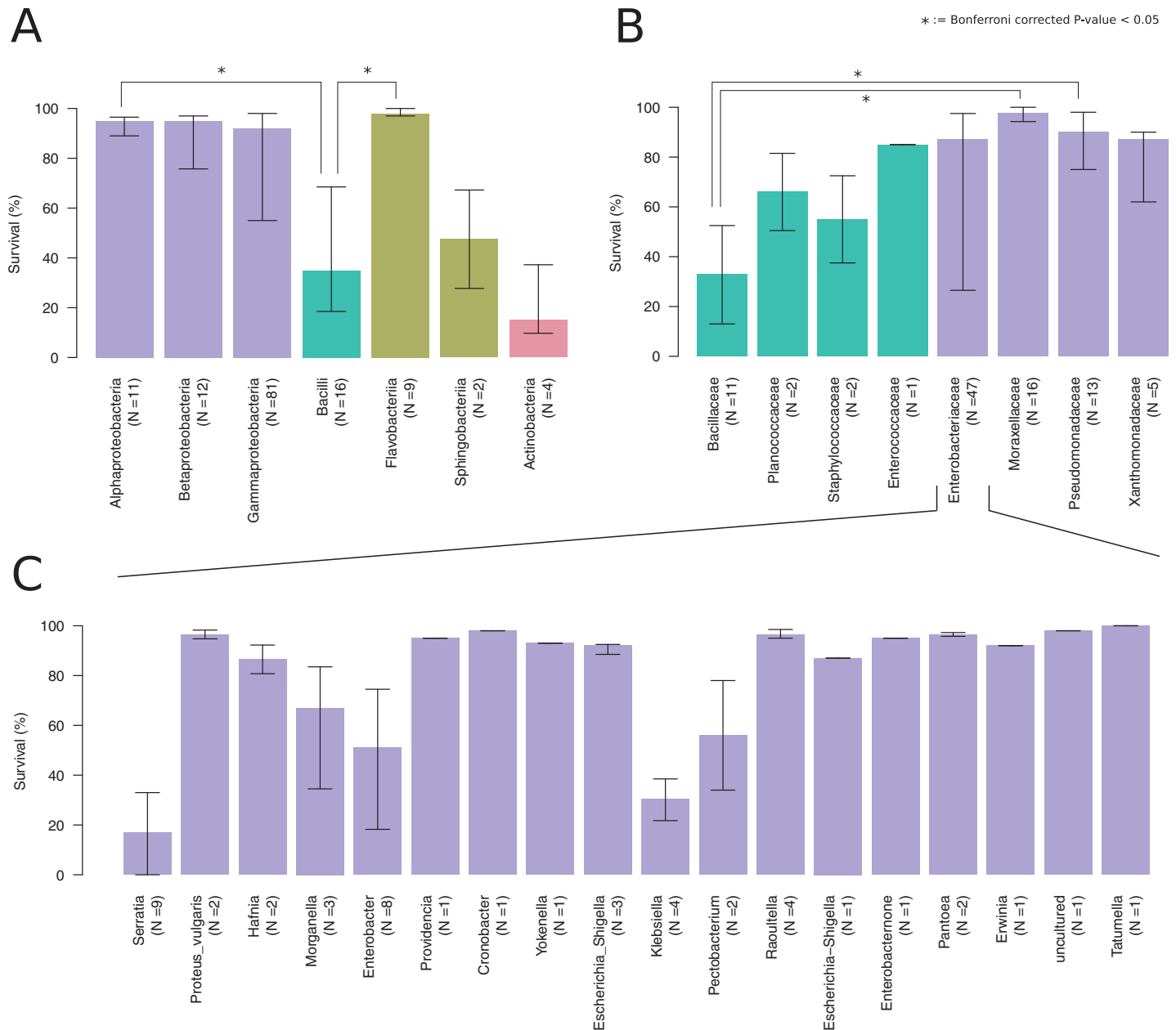
Fig 3. Survival of *P. pacificus* nematodes on different bacteria. (A) Bars show median and interquartile ranges of survival of *P. pacificus* worms in response to various bacterial classes. All pairwise tests for significantly different survival were done by a Wilcoxon rank-sum test. Results that remained significant after Bonferroni correction are highlighted. (B) Distribution of survival rate for deeply sampled and highly variable bacterial classes (Bacilli and Gammaproteobacteria) at higher phylogenetic resolution (family level). Within the class Bacilli, decreased survival is mostly due to strains of the family Bacillaceae. Within the class Gammaproteobacteria, the largest variability in nematode survival is observed in the family Enterobacteriaceae. (C) Distribution of survival within Enterobacteriaceae at genus level.

https://doi.org/10.1371/journal.pone.0198018.g003

pathogens, we calculated the correlation coefficients between chemotaxis and survival data (Fig 5A), which revealed only a weak trend for the whole data set (Spearman's rho = 0.154, P = 0.075). Even restricting the analysis to bacterial strains that were isolated from beetles and are therefore more likely to be seen by *P. pacificus* worms in the wild did not result in a higher correlation (Fig 5B). In particular, *P. pacificus* is attracted to multiple strains of the genus
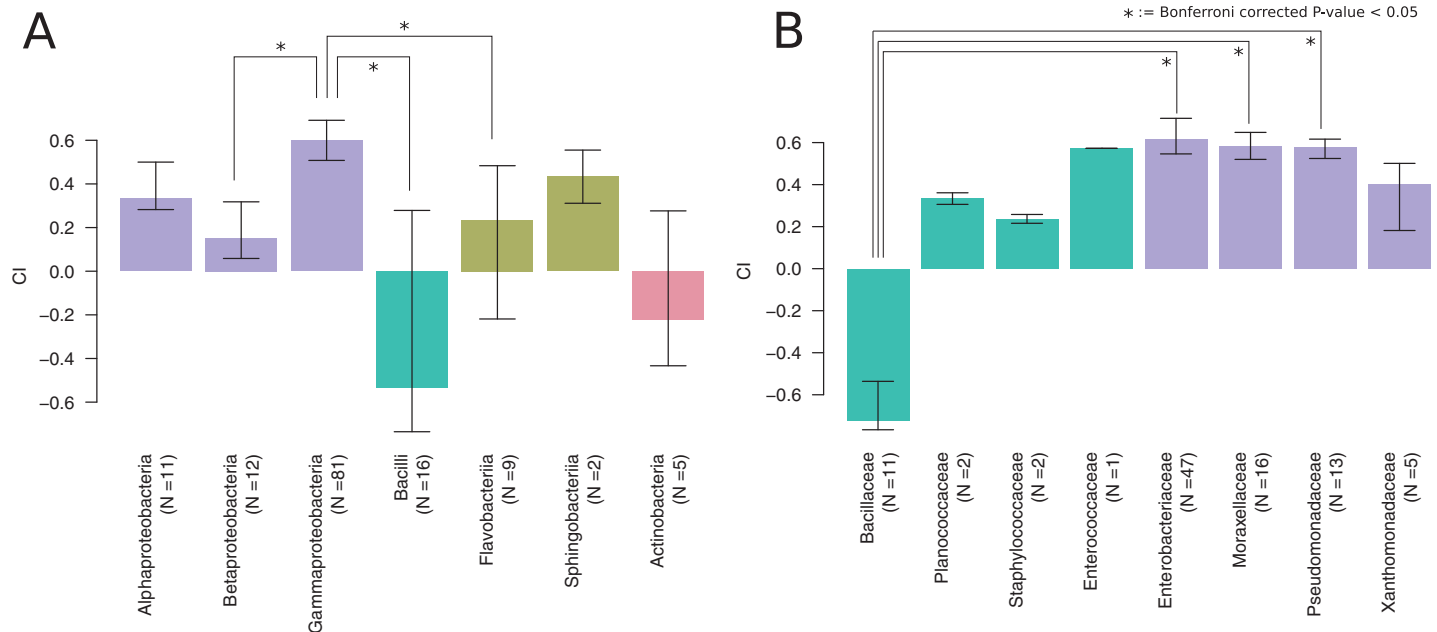
Fig 4. Chemoattraction towards different bacteria. (A) While most bacterial strains seem to be preferred over control spots, the class Bacilli shows a significantly repulsive effect (Wilcoxon test, Bonferroni corrected p-value<0.05) in comparison to three other bacterial classes. (B) Within the class Bacilli, the repulsive effect is largely due to strains of the family Bacillaceae.

Serratia, which are known pathogens of this nematode species (Figs 3C and 4). Thus, *P. pacificus* can recognize and avoid certain but not all pathogens.

Discussion

In this study we have isolated and characterized 136 bacterial strains from *Pristionchus*-associated environments, scarab beetles and figs. As ecologically relevant results of microbial-animal interactions are most likely to be obtained when microbes from the same environment are used in which the test organism lives, we only isolated bacteria from samples that showed the presence of *Pristionchus* nematodes. Despite the fact that our culture conditions most likely only allow isolation of a small percentage of the total bacterial community, the cultivable strains will form a powerful resource to study how *Pristionchus* nematodes interact with their environment and in particular, how bacterial diet can influence developmental decisions, such as the mouth form dimorphism [9]. Previous work on *Cryptococcus* yeast has demonstrated shifts in mouth form ratios of *P. pacificus* nematodes upon altered diet [15]. Thus, it is highly likely that some of the isolated bacteria induce similar effects.

We have screened *P. pacificus* survival on all isolated bacterial strains and found that multiple strains of diverse taxonomic groups are candidates for nematode pathogens. Among these, the genus *Serratia* has been previously described as potent killer of *P. pacificus* and *C. elegans* [30] and our survival assays showed that one of the *Stenotrophomonas* sp. isolates can be a potential pathogen to *P. pacificus*. Note that survival of nematodes on individual bacteria does not necessarily indicate the ability of the nematode to grow and reproduce on these strains. However, during the course of experiments, we kept nematodes on various bacterial strains for several generations indicating that *P. pacificus* can complete its life cycle on many of the isolated bacteria. It is important to note that in nature *P. pacificus* is exposed to a mixture of bacteria and therefore, survival assays performed with monoxenic cultures of test bacteria are
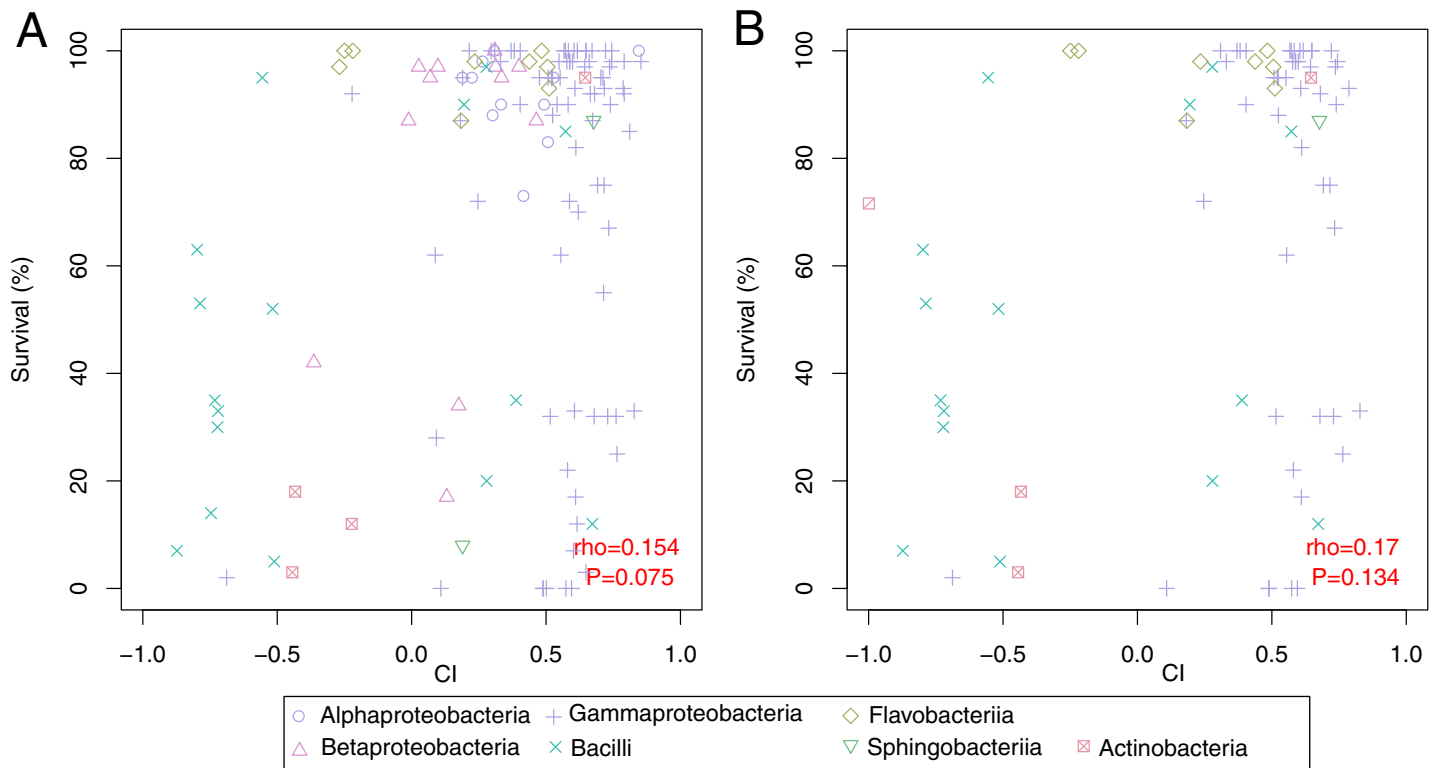
Fig 5. Correlation between survival and chemotaxis. (A) Testing for the correlation between survival and chemotaxis data we found a weak trend (Spearman's rho = 0.154, P = 0.075) for bacteria resulting in higher survival rates to also have higher chemotaxis indices, compared to strains that do not support growth or are pathogenic. (B) Similar correlation tests for those bacterial strains isolated from beetles where again, no strong signal was observed (Spearman's rho = 0.17, P = 0.134).

https://doi.org/10.1371/journal.pone.0198018.g005

partially artificial. Future studies will aim to study combinations of bacteria simultaneously, thereby mimicking more closely the situation seen in nature.

Complementary experiments of chemotaxis showed that bacterial classes like Bacilli and Actinobacteria that caused reduced survival are avoided by *P. pacificus*, which preferred feeding on *E. coli* OP50 when having the choice. However, explicitly testing for a correlation between survival and chemotaxis data did not allow the conclusion that *P. pacificus* can broadly recognize and avoid pathogenic bacteria. Overall, our chemotaxis experiments may suggest that most isolates are preferred over *E. coli* OP50 control spots. This finding came as a surprise given that the strain PS312 is in permanent culture since 1988 and has been exclusively fed on *E. coli* OP50 [5], but apparently has not developed a preference for it. Nevertheless this finding is consistent with observations from *C. elegans* showing that other bacteria such as *Comamonas* are much better food sources than *E. coli* OP50 [5,31]. However, the statement that *P. pacificus* prefers many bacteria over *E. coli* OP50 is to be regarded with care because the assay conditions utilized can not control for the exact bacterial concentration. In many of the assays, we observed that thicker colonies are not necessarily preferred by nematodes, suggesting that even if differences in bacterial concentrations exist, they seem to have a minor effect on the results of the chemotaxis assays. This might be due to the strong *P. pacificus* perception of oxygen [32]. Taken together, the survival and chemotaxis data showed substantial phylogenetic signal indicating that related bacteria give rise to a similar response in terms of nematode survival and chemoattraction. This may suggest that the overall biochemical composition of bacteria causes the observed effect on *P. pacificus* nematodes. Interestingly both potential groups of pathogens (Bacilli and Actinobacteria) that can be recognized and avoided by *P.*

pacificus are Gram-positive and spore forming bacteria suggesting that one or multiple features associated or correlated with the Gram-positive life style and/or spore formation are responsible for the response in nematodes.

In summary, the collection of bacterial strains that has been described in this study constitutes a resource for future studies of interactions between nematodes and bacteria. Our findings raise a number of interesting questions for future investigations, e.g. which bacterial factors are recognized by worms and how do they sense them? Given the substantial variability in survival, how are these patterns reflected in terms of development and other life history traits? Which of the isolated bacteria is the best food source for *P. pacificus*? Given that these associations can be studied in very controlled conditions and nematodes and bacteria are genetically tractable, combined investigation of nematodes and bacteria forms a powerful experimental system to study the effect of microbiota on organisms at a mechanistic level.

Supporting information

S1 Table. *Pristionchus*-associated bacteria strain names and sequence information. (XLSX)

Acknowledgments

We thank Drs. Vladislav Susoy, Matthias Herrmann, Eduardo Moreno for providing beetle and fig material.

Author Contributions

Conceptualization: Ralf J. Sommer.

Data curation: Nermin Akduman.

Formal analysis: Nermin Akduman, Christian Rödelsperger.

Funding acquisition: Ralf J. Sommer.

Investigation: Nermin Akduman.

Methodology: Nermin Akduman.

Project administration: Christian Rödelsperger, Ralf J. Sommer.

Supervision: Christian Rödelsperger, Ralf J. Sommer.

Visualization: Nermin Akduman, Christian Rödelsperger.

Writing – original draft: Nermin Akduman, Christian Rödelsperger.

Writing – review & editing: Nermin Akduman, Christian Rödelsperger, Ralf J. Sommer.

References

1. Althani AA, Marei HE, Hamdi WS, Nasrallah GK, El Zowalaty ME, Al Khodor S, et al. Human microbiome and its association with health and diseases. J Cell Physiol. 2016 Aug; 231(8):1688–94. https://doi.org/10.1002/jcp.25284 PMID: 26660761

2. Yilmaz LS, Walhout AJM. Worms, bacteria, and micronutrients: an elegant model of our diet. Trends Genet. 2014 Nov 26; 30(11):496–503. https://doi.org/10.1016/j.tig.2014.07.010 PMID: 25172020

3. Watson E, MacNeil LT, Ritter AD, Yilmaz LS, Rosebrock AP, Caudy AA, et al. Interspecies systems biology uncovers metabolites affecting *C. elegans* gene expression and life history traits. Cell. 2014 Feb 13; 156(4):759–70. https://doi.org/10.1016/j.cell.2014.01.047 PMID: 24529378

4. MacNeil L, Watson E, Arda HE, Zhu LJ, Walhout AJM. Diet-induced developmental acceleration independent of TOR and Insulin in *C. elegans*. Cell. 2013 Mar 28; 153(1): https://doi.org/10.1016/j.cell.2013.02.049 PMID: 23540701

5. Sommer RJ, Carta LK, Kim S, Sternberg PW. Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n.(Nematoda: Neodiplogasteridae) Fundam. Appl. Nematol., 1996, 19(6),511–521.

6. Dieterich C, Clifton SW, Schuster LN, Chinwalla A, Delehaunty K, Dinkelacker I,et al. The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. Nat Genet. 2008 Oct; 40(10):1193–8. https://doi.org/10.1038/ng.227 PMID: 18806794

7. Meyer JM, Baskaran P, Quast C, Susoy V, Rödelsperger C, Glöckner FO, et al. Succession and dynamics of *Pristionchus* nematodes and their microbiome during decomposition of *Oryctes borbonicus* on La Réunion Island. Environ Microbiol. 2017; 19(4):1476–89. https://doi.org/10.1111/1462-2920.13697 PMID: 28198090

8. Bento G, Ogawa A, Sommer RJ. Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. Nature. 2010 Jul 22; 466(7305):494–7. https://doi.org/10.1038/nature09164 PMID: 20592728

9. Ragsdale EJ, Müller MR, Rödelsperger C, Sommer RJ. A developmental switch coupled to the evolution of plasticity acts through a sulfatase. Cell. 2013 Nov 7; 155(4):922–33. https://doi.org/10.1016/j.cell.2013.09.054 PMID: 24209628

10. Rödelsperger C, Meyer JM, Prabh N, Lanz C, Bemm F, Sommer RJ. Single-molecule sequencing reveals the chromosome-scale genomic architecture of the nematode model organism *Pristionchus pacificus*. Cell Rep. 2017 Oct 17; 21(3):834–844. https://doi.org/10.1016/j.celrep.2017.09.077 PMID: 29045848

11. Wilecki M, Lightfoot JW, Susoy V, Sommer RJ. Predatory feeding behaviour in *Pristionchus* nematodes is dependent on phenotypic plasticity and induced by serotonin. J Exp Biol. 2015 May; 218:1306–13. https://doi.org/10.1242/jeb.118620 PMID: 25767144

12. Weitekamp CA, Libbrecht R, Keller L. Genetics and evolution of social behavior in insects. Annu Rev Genet Annual Reviews; 2017 Nov 27; 51(1):219–39.

13. Beldade P, Mateus ARA, Keller RA. Evolution and molecular mechanisms of adaptive developmental plasticity. Mol Ecol. 2011; 20(7):1347–63. https://doi.org/10.1111/j.1365-294X.2011.05016.x PMID: 21342300

14. Werner MS, Sieriebriennikov B, Loschko T, Namdeo S, Lenuzzi M, Dardiry M, et al. Environmental influence on *Pristionchus pacificus* mouth form through different culture methods. Sci Rep. 2017 Aug 3; 7:7207. https://doi.org/10.1038/s41598-017-07455-7 PMID: 28775277

15. Sanghvi G V, Baskaran P, Röseler W, Sieriebriennikov B, Rödelsperger C, Sommer RJ. Life history responses and gene expression profiles of the nematode *Pristionchus pacificus* cultured on *Cryptococcus* yeasts. PLoS One. 2016 Oct 14; 11(10):e0164881. https://doi.org/10.1371/journal.pone.0164881 PMID: 27741297

16. Ragsdale E J, Kanzaki N, Herrmann M. Taxonomy and natural history: the genus *Pristionchus*. In: *Pristionchus pacificus*. A nematode model for comparative and evolutionary biology. (Ed.: Sommer R J) Brill, Leiden.

17. Susoy V, Herrmann M, Kanzaki N, Kruger M, Nguyen CN, Rödelsperger C, et al. Large-scale diversification without genetic isolation in nematode symbionts of figs. Sci Adv. 2016 Jan 15; 2(1):e1501031. https://doi.org/10.1126/sciadv.1501031 PMID: 26824073

18. Rae R, Riebesell M, Dinkelacker I, Wang Q, Herrmann M, Weller AM, et al. Isolation of naturally associated bacteria of necromenic *Pristionchus* nematodes and fitness consequences. J Exp Biol. 2008; 211(12):1927–36.

19. Iatsenko I, Boichenko I, Sommer RJ. Bacillus thuringiensis DB27 produces two novel protoxins, Cry21Fa1 and Cry21Ha1, which act synergistically against nematodes. Appl Environ Microbiol. 2014 May; 80(10):3266–75. https://doi.org/10.1128/AEM.00464-14 PMID: 24632254

20. Iatsenko I, Sinha A, Rödelsperger C, Sommer RJ. New role for DCR-1/dicer in *Caenorhabditis elegans* innate immunity against the highly virulent bacterium Bacillus thuringiensis DB27. Infect Immun. 2013 Oct; 81(10):3942–57. https://doi.org/10.1128/IAI.00700-13 PMID: 23918784

21. Lightfoot JW, Chauhan VM, Aylott JW, Rödelsperger C. Comparative transcriptomics of the nematode gut identifies global shifts in feeding mode and pathogen susceptibility. BMC Res Notes. 2016 Mar 5; 9:142. https://doi.org/10.1186/s13104-016-1886-9 PMID: 26944260

22. Rae R, Witte H, Rödelsperger C, Sommer RJ. The importance of being regular: *Caenorhabditis elegans* and *Pristionchus pacificus* defecation mutants are hypersusceptible to bacterial pathogens.Int J Parasitol. 2012 Jul; 42(8):747–53. https://doi.org/10.1016/j.ijpara.2012.05.005 PMID: 22705203

23. McGaughran A, Rödelsperger C, Grimm DG, Meyer JM, Moreno E, Morgan K, et al. Genomic profiles of diversification and genotype-phenotype association in island nematode lineages. Mol Biol Evol. 2016; 33(9):2257–72. https://doi.org/10.1093/molbev/msw093 PMID: 27189551

24. Stiernagle T. Maintenance of *C. elegans*.WormBook. 2006 Feb 11:1–11

25. Herrmann M, Mayer WE, Hong RL, Kienle S, Minasaki R, Sommer RJ. The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. Zoolog Sci. 2007; 24(9):883–9. https://doi.org/10.2108/zsj.24.883 PMID: 17960992

26. Sambrook J., Fritsch E. F. and Maniatis T. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Press; 1989.

27. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013 Jan 27; 41 (Database issue):D590–6. https://doi.org/10.1093/nar/gks1219 PMID: 23193283

28. Zhang Y, Lu H, Bargmann CI. Pathogenic bacteria induce aversive olfactory learning in *Caenorhabditis elegans*. Nature. 2005; 438(7065):179–84. https://doi.org/10.1038/nature04216 PMID: 16281027

29. Hong RL, Sommer RJ. Chemoattraction in *Pristionchus* nematodes and implications for insect recognition. Curr Biol. 2006; 16(23):2359–65. https://doi.org/10.1016/j.cub.2006.10.031 PMID: 17141618

30. Sinha A, Rae R, Iatsenko I, Sommer RJ. System wide analysis of the evolution of innate immunity in the nematode model species *Caenorhabditis elegans* and *Pristionchus pacificus*. PLoS One. 2012; 7(9).

31. Shtonda BB, Avery L. Dietary choice behavior in *Caenorhabditis elegans*. J Exp Biol. 2006 Jan; 209(Pt 1)89–102. https://doi.org/10.1242/jeb.01955 PMID: 16354781

32. Moreno E, McGaughran A, Rödelsperger C, Zimmer M, Sommer RJ. Oxygen-induced social behaviours in *Pristionchus pacificus* have a distinct evolutionary history and genetic regulation from *Caenorhabditis elegans*. Proc Biol Sci. 2016 Feb 24; 283(1825):20152263. https://doi.org/10.1098/rspb.2015.2263 PMID: 26888028

3.2. Predatory behaviors are enhanced by microbiota derived vitamin B12 in nematodes

Nermin Akduman, Christian Rödelsperger, James W. Lightfoot, Waltraud Röseler, Hanh Witte, Wen-Sui Lo, Ralf J. Sommer, submitted for publication

3.2.1. Contribution table

Author	Author position	Scientific ideas %	Data generation %	Analysis & Interpretation %	Paper writing %
Nermin Akduman	First	60	60	80	25
Dr. Christian Rödelsperger	Second	25	10	10	30
Dr. James Lightfoot	Third	15	10	10	45
Waltraud Röseler	Fourth	0	10	0	0
Hanh Witte	Fifth	0	5	0	0
Dr. Wen-Sui Lo	Sixth	0	5	0	0
Title of paper:		Predatory behaviors are enhanced by microbiota derived vitamin B12 in nematodes			
Status in publication process:		Submitted in 2019			

Bacterial derived vitamin B12 enhances predatory behaviors in nematodes

Nermin Akduman[1], James W. Lightfoot[1], Waltraud Röseler[1], Hanh Witte[1], Wen-Sui Lo[1], Christian Rödelsperger[1], Ralf J. Sommer[1]*

*corresponding author: ralf.sommer@tuebingen.mpg.de

Affiliations: [1]Department for Evolutionary Biology, Max Planck Institute for Developmental Biology, Max Planck Ring 9, 72076 Tübingen Germany

KEYWORDS

Microbiome, microbiota, diet, surplus killing, metabolism, development, *Pristionchus pacificus*, *Caenorhabditis elegans*

The microbiome is known to affect host development, metabolism and immunity, however, its impact on behaviors is only beginning to be understood. Here, we investigate how bacteria modulate complex behaviors in the nematode model organism *Pristionchus pacificus. P. pacificus* is a predator feeding on the larvae of other nematodes including *Caenorhabditis elegans.* Growing *P. pacificus* on different bacteria and testing their ability to kill *C. elegans* reveals drastic differences in killing efficiencies with a *Novosphingobium* species showing the strongest enhancement. Strikingly, increased killing was not accompanied by an increase in feeding, a phenomenon known as surplus-killing whereby predators kill more prey than necessary for sustenance. RNA-seq revealed widespread metabolic rewiring upon exposure to *Novosphingobium*, which facilitated the screening for bacterial mutants leading to an altered transcriptional response. This identified bacterial derived vitamin B12 as a major micronutrient enhancing predatory behaviors. Vitamin B12 is an essential cofactor for detoxification and metabolite biosynthesis and has previously been shown to accelerate development in *C. elegans*. In *P. pacificus* vitamin B12 supplementation amplified, whereas mutants in vitamin B12-dependent pathways reduced surplus-killing. This demonstrates that bacterial vitamin B12 affects complex behaviors and thus establishes a connection between microbial diet and the nervous system.

The microbiome is considered a fundamental aspect of a host's biology and is known to provide developmental cues, influence metabolism and alter immunity[1-3]. However, the microbiome constitutes a complex network of microorganisms and disentangling specific interactions and effects at a mechanistic level is challenging. Bacterial-feeding nematodes constitute a highly attractive system to study the influence of the microbiome because specific interactions can be investigated in monoxenic cultures where the microbiome and diet are indistinguishable from one another and easily controlled. To study the effect of bacteria on behavior we investigate the nematode model organism *Pristionchus pacificus* that exhibits a particular complex behavior unknown from *C. elegans*. In general, *P. pacificus* is an omnivorous nematode that can grow on bacteria, fungi and it can predate on other nematodes [4-6]. Predation is dependent on morphological and behavioral novelties, involving the formation of teeth-like denticles and a self-recognition mechanism[7-10]. The ability to form teeth-like denticles is an example of developmental plasticity with two discrete mouth-forms[11]. The stenostomatous morph has a single blunt tooth, whereas the eurystomatous morph has two large teeth with only the latter capable of predation (Fig. 1A and B)[7]. Predation may confer a selective advantage in certain environmental settings with previous studies indicating that different culture conditions, including microbial diet, are able to modulate the ratio of the two mouth forms[12,13]. Furthermore, *P. pacificus* predation under laboratory conditions is also an example of a phenomenon known as surplus-killing behavior[6]. Surplus-killing is a well-documented complex behavior observed in many predators across the animal kingdom, in which more prey are killed than nutritionally required[14-22]. Theoretical and experimental studies considered surplus-killing a potentially context-dependent, adaptive foraging strategy or alternatively, a context-general syndrome of high aggression[15,17,20-23]. However, the full impact of diet on killing and predation is currently poorly understood.

Therefore, we tested the effect of 25 different bacteria recently isolated from *Pristionchus*-associated environments[24] on various predation associated traits. Specifically, we grew *P. pacificus* for several generations on monoxenic cultures and investigated the effect on mouth form ratio, pharyngeal pumping, and killing behavior by comparing them to standard laboratory cultures grown on *Escherichia coli* OP50. While diet had a limited effect on mouth form ratios and pharyngeal pumping, we found up to a four-fold difference in killing efficiency depending on microbial diet (Fig. 1C, fig. S1A and B). The strongest effect on killing efficiency was observed when *P. pacificus* was fed upon three alpha-proteobacteria of the genera *Novosphingobium* and *Rhizobium*, resulting in up to 160 corpses of dead prey in standardized corpse

3

69   assays (Fig. 1C). We therefore focused on one bacterium of this group,

70   *Novosphingobium* L76.

71   Stronger killing efficiency translated into higher rates of surplus-killing.

72   Specifically, we performed bite assays to observe individual predators for 10 minutes

73   to distinguish specific predatory events including biting, successful biting that results

74   in penetration of the cuticle, and feeding on prey larvae (see Method section for exact

75   description of terms). When grown on *E. coli* OP50, *P. pacificus* only kills 50% of its

76   prey after biting, and subsequent feeding was only observed in roughly 10% of all

77   cases (Fig. 1D, Movie S1). Using *Novosphingobium* L76, we found that the number

78   of *P. pacificus* bites and successful biting events indeed doubled relative to *E. coli*

79   OP50 grown predators (Fig. 1D). However, we found no increase in feeding on the

80   dead prey (Fig. 1D). Instead, predators rapidly moved over agar plates searching for

81   new prey items. Thus, a *Novosphingobium* diet enhances predation and surplus-

82   killing.

83   Next, we established the necessary bacterial exposure time required to

84   influence predatory behavior and additionally, wanted to know whether the increase

85   in killing was mediated by factors secreted by the bacteria or solely by their ingestion.

86   Only a limited exposure to a diet of *Novoshingobium* L76 during development was

87   sufficient for *P. pacificus* nematodes to exhibit increased predatory behavior,

88   however, *Novosphingobium* L76 culture supernatants alone were unable to

89   recapitulate this effect (Fig. 1E, fig. S1C). In contrast, when *Novosphingobium* was

90   diluted with *E. coli* OP50, the effect still persisted suggesting that the response to

91   *Novosphingobium* L76 is unlikely due to differences in caloric intake (fig. S1D).

92   Instead, the behavioral change is likely a result of physiological alterations caused by

93   the different nutritional composition of *Novosphingobium* L76. Therefore, we

94   analyzed the transcriptomic response of young *P. pacificus* adults grown on

95   *Novosphingobium* in comparison with *E. coli*. We identified a total of 2,677 (9%)

96   genes with significant differential expression (FDR corrected P-value < 0.05)

97   between the two bacterial diets (Table S1). Most strikingly, more than half of all

98   genes that are predicted to be involved in fatty acid metabolism are significantly

99   differentially expressed between the two diets (Fig. 2A and B).

100   To study the mechanisms by which *Novosphingobium* alters fatty acid

101   metabolism and induces behavioral changes in the nematode, we used an unbiased

102   bacterial mutagenesis approach. We replaced *Novosphingobium* L76 with

103   *Novosphingobium lindaniclasticum* LE124 (*N. lin.* LE124 thereafter), as the latter can

104   easily be manipulated by transposon mutagenesis, has an available genome[25], and

105   induces similar behavioral effects in *P. pacificus* (fig. S1E). Additionally, to detect any

physiological changes in *P. pacificus* caused by mutations in the bacteria, two dietary sensors were generated using *P. pacificus* fatty acid metabolism genes that showed differential expression on different bacteria (Fig 2A and B). Specifically, we used homologs of the acyl-CoA synthetase enzyme *Ppa-acs-19.1,* which was upregulated on *E. coli* OP50 and downregulated on *Novosphingobium,* as well as the short-chain dehydrogenase reductase enzyme *Ppa-stdh-1,* which has the opposite expression profile (Fig. 2C and D, fig. S2). Both reporter lines confirmed the differential expression that was detected by RNA-seq with *Ppa-acs-19.1* being expressed nearly exclusively on *E. coli*, whereas *Ppa-stdh-1* is expressed highly on *Novosphingobium* but only minimally on *E. coli* OP50 (Fig. 2C and D). Subsequently, we used these dietary sensors to screen for bacterial mutants that fail to differentially regulate these genes. From a library of 4,320 *N. lin.* LE124 mutants, three affected the expression of *Ppa-stdh-1* and 21 altered the expression of *Ppa-acs-19.1*. Whole genome sequencing of these bacterial mutants identified transposon insertions in genes corresponding to four biological pathways: purine and pyrimidine metabolism, nitrogen metabolism, and vitamin B12 (Fig. 2E; fig. S2C, key resources table). Importantly, in mutants of all four pathways, the change of transcriptomic response coincided with a reduction in predatory behavior including surplus-killing relative to wild-type *N. lin.* LE124 (Fig. 2F, fig. S2D and E). Thus, the dietary sensor allows the identification of factors regulating complex behavioral traits.

Vitamin B12 has been shown to be a crucial co-factor involved in growth, development and behavior in several animals, including mice and human[26]. Therefore, we focus on vitamin B12, which was recently also found to affect growth and development of *C. elegans*[27], whereas nothing is known about vitamin B12 affecting *C. elegans* behavior. We first analyzed if vitamin B12 supplementation was sufficient to affect the expression of the *Ppa-acs-19.1* sensor and determined the required concentration for this. Supplementation of an *E. coli* diet with 500nM vitamin B12 resulted in the absence of *Ppa-acs-19.1* expression with no adverse effects to the health of wild-type animals (fig. S3A). Additionally, this vitamin B12 concentration abolished *Ppa-acs-19.1* expression on *N.lin.*LE124 *CbiQ::Tn5* mutants (fig. S3B). Subsequently, we analyzed if this supplementation was also sufficient to enhance the predatory behaviors. Indeed, supplementation with 500nM vitamin B12 rescued the vitamin B12-deficient *N. lin.* LE124 *CbiQ* mutant and similarly, increased surplus-killing behavior on an *E. coli* diet (Fig. 3A and B). These results demonstrate that vitamin B12 is an important micronutrient involved in complex behaviors in nematodes.

142   Studies by Walhout and co-workers in *C. elegans* showed that developmental

143   acceleration under a *Comamonas aq.* DA1877 diet was also due to vitamin B12[27].

144   Given the similarities of the *C. elegans* developmental response to *Comamonas*

145   DA1877 and the behavioral response of *P. pacificus* to *N. lin.* LE124, we compared

146   the effect of both bacteria on development and behavior.   Indeed, *Comamonas*

147   DA1877 as well as *N. lin.* LE124 induced developmental acceleration of *C. elegans*

148   and *P. pacificus* (Fig. 3C). Similarly, both bacteria enhanced predatory behaviors of

149   *P. pacificus* (Fig. 3D). Thus, the differential effect of bacterial diet on nematode

150   development and behavior might often be due to the uneven distribution of vitamin

151   B12 biosynthesis capabilities of bacteria.

152   In many animals and humans, vitamin B12 is a co-factor for two enzymes in

153   different pathways (fig. S4A). Methionine-synthase (MS) converts homocysteine to

154   methionine in the cytosolic methionine/S-adenosylmethionine (SAM) cycle and in *C.*

155   *elegans* is encoded by the *metr-1* gene. The second enzyme, methylmalonyl

156   coenzyme A (CoA) mutase, converts methylmalonyl-CoA to succinyl-CoA in

157   mitochondria and is encoded by the *mce-1* gene in *C. elegans*. In humans, vitamin

158   B12 deficiency causes methylmalonic aciduria and homocysteinemia resulting in

159   devastating diseases[28]. To test if both pathways are required for increased killing

160   behavior in *P. pacificus*, we generated CRISPR/Cas9-derived mutants in *Ppa-metr-1*

161   and *Ppa-mce-1* (fig. S4B, C and D). Both mutants failed to respond to the

162   supplementation of an *E. coli* diet with vitamin B12 (Fig. 4A). Given that SAM is a

163   donor of methyl-groups for many different substrates including RNA, DNA, and

164   proteins, we supplemented an *E. coli* diet of *P. pacificus* wild type and *Ppa-metr-1*

165   mutant animals with methionine. In both cases, methionine supplementation resulted

166   in enhanced killing behavior (Fig. 4B). Thus, both vitamin B12-dependent pathways

167   seem to be involved in *P. pacificus* predatory behaviors.

168   The experiments described above indicate crucial roles of bacterial derived

169   vitamin B12 for the development and behavior of both *P. pacificus* and *C. elegans*.

170   As these nematodes are estimated to have diverged roughly 100 Mya[29], we next

171   tested how prevalent the effects of vitamin B12 are on the development and

172   physiology of other nematodes, including more distantly related species and

173   representatives that live in diverse ecological settings (supplementary table 1). We

174   grew six nematode species of four major taxonomic clades on a vitamin B12

175   supplemented diet and measured the effects on their development and growth by

176   quantifying the total worm volume of young adults. In all species tested, we found a

177   significant increase in worm volume (Fig. 4C and D). This included the facultative

178   parasite *Parastrongyloides   trichosuri*   and   the   entomopathogenic   nematode

6

179 *Steinernema carpocapsae*. We found the strongest effect on the large free-living
180 nematode *Allodiplogaster sudhausi* that nearly doubled its volume on a vitamin B12
181 supplemented diet (Fig. 4D). Where possible, we also investigated the effects on
182 developmental speed. Similar to the increase in body size, vitamin B12
183 supplementation accelerated the development of *Rhabditophanes* sp. and *A.*
184 *sudhausi* (fig. S4E and F). Taken together, these results demonstrate important
185 physiological and developmental functions of vitamin B12 that are shared across
186 many nematode species.

187   Our study identified a novel role for nematode-associated microbiota in
188 modulating the complex behavioral trait of predation and therefore, demonstrates a
189 connection between the microbial diet and the nervous system in nematodes.
190 Diverse bacterial species had different effects on the predatory behavioral state with
191 some adversely influencing predation while others enhanced the predatory
192 behaviors. The greatest enhancement in predatory behaviors was observed when *P.*
193 *pacificus* was fed upon *Novosphingobium* with this increase in killing influenced by
194 bacterial derived vitamin B12. Additionally, we have revealed a more general,
195 conserved role for vitamin B12 in nematode development and growth. Previous
196 studies have shown vitamin B12 to be essential for *C. elegans* development with
197 infertility, growth retardation and a reduction in life-span observed in animals deficient
198 in vitamin B12[27,30,31]. In contrast, behavioral effects have not been reported and
199 similarly, mechanisms of vitamin B12 deficiency in humans that result in
200 neuropathies are currently unknown. It is important to note that the modulation of
201 predation and surplus-killing in *P. pacificus* requires both vitamin B12-dependent
202 pathways. Therefore, we speculate that the influence of vitamin B12 on these
203 behaviors is multifactorial and might well involve several factors. Specifically, the
204 SAM pathway feeds into the methylation of DNA, RNA and proteins, but also lipids
205 and neurotransmitters (fig. S4a). Thus, the presence of vitamin B12 might act
206 through multiple downstream factors, but how it stimulates these effects has yet to be
207 discovered. Importantly however, several neural circuits and neurotransmitter
208 systems of *P. pacificus* have been investigated[6,32-34]. Therefore, future studies can
209 reveal the cellular and molecular foci of vitamin B12-dependence and the influence of
210 the microbiota on nematode predatory behaviors.

211 **METHODS**

212 **Nematode and Bacterial Strains**

213 A list of all nematode and bacterial species and strains can be found in key resources
214 table.

215 **Bacterial Culture Conditions**

216 All bacterial strains and mutants were grown overnight in LB (Lysogeny broth)
217 supplemented with 50μg/ml kanamycin where required. Bacteria were grown at 30°
218 C or 37° C depending on the species and 6 cm nematode growth medium (NGM)
219 plates were seeded with 50μl bacterial overnight cultures and were incubated for two
220 days.

221 **Nematode Culture Conditions**

222 *P. pacificus*, *C. elegans*, *Rhabditophanes* sp. KR302 and *A. sudhausi* were grown
223 under standard nematode growth conditions on NGM plates seeded with *Escherichia*
224 *coli* OP50. Egg cultures were obtained by treating healthy gravid adults with alkaline
225 hypochlorite (bleaching) and were maintained and raised at 20° C on NGM plates.
226 The free-living generation of *Parastrongyloides trichosuri* was cultured as described
227 in Grant et al (2006)[35]. Briefly, to maintain the *P. trichosuri* free-living generation in
228 culture, *E. coli* OP50-spotted NGM plates were incubated for two days at room
229 temperature (RT). Autoclaved rabbit feces were lightly broken and placed on the
230 spotted NGM plate along with *P. trichosuri* animals. Additional *E. coli* OP50
231 (supplemented with/without vitamin B12) was subsequently added to the dry rabbit
232 feces. The entomopathogenic nematode *Steinernema carpocapsae* was grown on its
233 symbiotic bacterium *Xenorhabdus nematophila*. Symbiotic bacteria were inoculated
234 in LB and incubated at 25°C overnight, 300μl from overnight cultures were spotted to
235 NGM plates (supplemented with/without vitamin B12) and incubated for 1 day at RT.
236 *S. carpocapsae* nematodes were transferred to their respective symbiotic bacterial
237 plates and subsequently grown at 20° C.

8

Mouth-form phenotyping

Mouth-form phenotyping was performed as previously reported[6,33]. In brief, axenic worm eggs were obtained by treating healthy gravid *P. pacificus* adults with alkaline hypochlorite, which were subsequently maintained on the test bacteria strains or mutants for at least two generations. Synchronized J4 larvae were picked onto NGM plates with the same test bacteria and roughly 12 hours (hrs) later, worms became young adults. NGM plates with synchronized young adults were placed onto a stereomicroscope with high magnification (150X). The eurystomatous (Eu) mouth form was determined by the presence of a wide mouth, whereas the stenostomatous (St) forms were determined by a narrow mouth. Eu young adult worms were picked for predation assays.

Predation assays:

We used two types of predation assays as described below.

Corpse assays

9

252   Corpse assays facilitated rapid quantification of predatory behavior and were

253   conducted as previously described[6,10,33]. Briefly, in order to generate substantial *C.*

254   *elegans* larvae for use as prey, cultures were maintained on *E. coli* OP50 bacteria

255   until freshly starved resulting in an abundance of young larvae. These plates were

256   washed with M9 buffer, passed through two Millipore 20µm filters and centrifuged at

257   377x g to form a concentrated larval pellet. Excess buffer was removed and 1µl of

258   worm pellet was deposited onto a 6 cm NGM unseeded assay plates. This resulted in

259   roughly 3000 prey larvae on each assay plate. Assay plates were left for a minimum

260   of one hour (h) to allow larvae to distribute evenly over the plate. Young adult *P.*

261   *pacificus* predators were screened for the predatory Eu mouth form and transferred

262   to empty NGM plates for 30 minutes (min) to remove any excess bacteria from their

263   bodies. Subsequently, five *P. pacificus* nematodes were added to each assay plate.

264   Predators were permitted to feed on the prey for two hrs before removal and the

265   plate was subsequently screened for the presence of larval corpses which were

266   identified by the absence of motility coinciding with obvious morphological defects

267   including leaking innards or missing worm fragments. Each assay was replicated ≥5

268   times. When post-feeding size measurement was required, predatory animals were

269   picked to NGM plates containing no bacteria and measurements were taken using

270   the Wormsizer plug in for Image J/Fiji [36]. See below for Wormsizer experimental

271   details.

272   **Bite assays**

273 Bite assays provide a more detailed and thorough analysis of the specific interactions
274 associated with predatory behaviors. Bite assays were conducted as previously
275 described[6,10]. Briefly, substantial *C. elegans* prey was generated by maintaining *C.*
276 *elegans* cultures on *E. coli* OP50 bacteria until freshly starved resulting in an
277 abundance of young larvae. These plates were washed with M9 buffer, passed
278 through two Millipore 20µm filters and centrifuged at 377x g to form a concentrated
279 larval pellet. Excess buffer was removed and 1µl of worm pellet was deposited onto a
280 6 cm NGM unseeded assay plate. This resulted in roughly 3000 prey larvae on each
281 assay plate. Assay plates were left for a minimum of one h to allow larvae to
282 distribute evenly over the plate. Young adult *P. pacificus* predators were screened for
283 the appropriate predatory Eu mouth morph and transferred to empty NGM plates for
284 30 min to remove any excess bacteria from their bodies. A single predator was
285 placed on to the assay plate and allowed to recover for 20 min. After recovery, the
286 predatory animal was directly observed under a light stereomicroscope for 10 min
287 and the number of bites, successful bites and feeding events quantified. "Bites" were
288 characterized by a switch to the slower predatory pharyngeal pumping rhythms
289 previously described[6,33] coinciding with a restriction in movement of the prey.
290 "Successful bites" were characterized by successful rupturing of the prey cuticle
291 resulting in sufficient damage to cause the innards to leak from the wound. "Feeding"
292 was characterized by consumption of the prey through either the observation of
293 prolonged predatory feeding rhythms once the predator had successful grasped its
294 prey, or alternatively, observation of the faster bacterial associated feeding rhythms
295 at the site of a puncture wound. In these assays, no distinction was made as to
296 whether the predatory behavior events were against live prey or against recently
297 killed or wounded animals. Indeed, predators were occasionally observed repeatedly
298 biting the same dying or dead larvae and each contact was quantified as a distinct
299 predatory event. Each assay was conducted with 10 different animals.

300 **Pharyngeal pumping analysis**

11

301 *P. pacificus* worms were maintained on 6cm NGM agar plates and fed on the

302 appropriate test bacterial strains prior to assaying. Young adults were transferred

303 onto assay plates and allowed to recover for 15 min from the stress of being

304 transferred. Worms were observed on a Zeiss microscope at 40-63X magnifications,

305 with a high-speed camera and pharyngeal pumping was recorded for 15 seconds, at

306 50 Hz in at least 20 animals to ensure accurate quantification. The recorded movies

307 were replayed at the desired speed to count individual pumps as previously

308 described[6].

309 **E. coli OP50 supplementation with *Novosphingobium* L76 supernatant**

310 *E. coli* OP50 and *Novosphingobium* L76 were grown overnight in LB at 37° C and

311 30° C, respectively. 5ml overnight cultures of each bacteria were grown until they

312 measured an OD_{600} 1. Bacterial cultures were centrifuged at 10000 rpm, RT for 5 min

313 and supernatants were isolated by filtering with 5μm filters. The *E. coli* OP50 pellet

314 was re-suspended with 5ml *Novosphingobium* L76 supernatant. 300μl of the *E. coli*

315 OP50 with *Novosphingobium* L76 supernatant was subsequently spotted to 6 cm

316 NGM plates. OP50 pellet with OP50 supernatant and additionally, *Novosphingobium*

317 L76 were also spotted to 6 cm NGM plates as controls. Spotted NGM plates were

318 ready for assay after two days of incubation. Freshly bleached eggs from well-grown

319 *P. pacificus* cultures were then transferred onto assay plates and worms were

320 transferred to new assay plates two days later. Worms were grown until young adult

321 stage and synchronized young adults were picked and assessed via corpse assays.

322 **Mixing Bacterial Diets**

323 Liquid cultures of *E. coli* OP50 and *Novosphingobium* L76 were grown in LB at 37° C

324 and 30° C, respectively. Bacterial cultures were diluted to the same OD_{600} and mixed

325 in ratios 1/10,1/100 and 1/1000. Bacterial suspensions were spread onto peptone-

326 free NGM plates to minimize bacterial growth and plates were briefly air dried in a

327 sterile hood. Bleached *P. pacificus* eggs were added to the plates and worms were

328 allowed to grow until young adult stage; synchronized young adults were then picked

329 and assessed via corpse assays.

330 **Switching bacterial diet**

331 Overnight cultures of *E. coli* OP50 and *Novosphingobium* L76 were spread to NGM

332 plates and incubated at RT for two days. Subsequently, bleached *P. pacificus* eggs

333 were added to the *E. coli* OP50 plates. Worms were transferred from these *E. coli*

334 OP50 plates to *Novosphingobium* L76 at specific developmental stages, L2, L3 and

335 L4, respectively, and were allowed to develop into young adult stage on

336 *Novosphingobium* L76. Worms fed with *E. coli* OP50 or *Novosphingobium* L76 from

337 egg to young adult stage were used as controls. Synchronized young adults were

338 then picked and assessed via corpse assays.

339 **RNA sequencing**

340 Bacterial strains were grown in LB overnight and spotted to 6 cm NGM plates.

341 Starting from bleached eggs *P. pacificus* nematodes were grown on bacteria for at

342 least two generations and 50 young adults were picked for RNA isolation. Total RNA

343 was extracted using Direct-Zol RNA Mini prep kit (Zymo Research) according to the

344 manufacturer's guidelines. RNA libraries were prepared by following Truseq RNA

345 library prep kit according to the manufacturer's guidelines from 1µg of total RNA in

346 each sample (Illumina Company). Libraries were quantified using a combination of

347 Qubit and Bioanalyzer (Agilent Technologies) and normalized to 2.5nM. Samples

348 were subsequently sequenced as 150 bp paired end reads on multiplexed lanes of

349 an Illumina HiSeq3000 (IIlumina Inc). Raw reads have been uploaded to the

350 European Nucleotide archive under the study accession PRJEB33410.

351 **Analysis of RNA-seq data**

352 The software TopHat (version:2.0.14) was used to align raw reads against the *P.*

353 *pacificus* reference genome (pristionchus.org, version: Hybrid1) and tests for

354 differential expression were performed by Cuffdiff (version: 2.2.1)[37]. Genes with an

355 FDR-corrected p-value < 0.05 were considered as significantly differentially

356 expressed. For up and downregulated genes, the most significantly enriched

357 metabolic pathways were identified as described previously[12].

358 **Generation of transgenic lines**

13

359   We selected the genes *Ppa-stdh-1* and *Ppa-acs-19.1* to generate transcriptional

360   reporters and established transgenic lines necessary for their use as dietary sensors*.*

361   For *Ppa-stdh-1,* a 2.3 kb interval encompassing the upstream region and the first two

362   exons was amplified. For *Ppa-acs-19.1,* a 1.4 kb region upstream of the first

363   predicted exon was amplified. These promoters were fused to TurboRFP (Evrogen),

364   together with the 3′ UTR sequence of the gene *Ppa-rpl-23* using the following

365   overlapping primers

366   *Ppa-stdh-1 -* F:

367   5'-GCCAAGCTTGCATGCCTGCACATGCTATGGAGCGTAGC-3';

368   *Ppa-stdh-1 -* R:

369   5'-CTGAAAAAAAAAACCCAAGCTTGGGTCCCGAAGACGACGTTGTAGAC-3';

370   *Ppa-acs-19.1 -*F

371   5'-GGATCCCGTCGACCTGCAGGCATG-3';

372   *Ppa-acs-19.1 –*

373   R 5'-ATGAGCGAGCTGATCAAG-3';

374   *TurboRFP -*F

375   5'- TGCATGCCTGCAGGTCGACGGGATCCGCCATCACTATGCATTGCTG-3' and

376   *TurboRFP-* R

377   5'-TCCTTGATCAGCTCGCTCATCTGAACCAGCAAGGGCGATAG-3'.

378   PCR fragments were assembled using Gibson assembly kit (NEB) and verified by

379   Sanger sequencing. The *Ppa-stdh-1*::RFP and *Ppa-acs-19.1*::RFP constructs were

380   amplified with the addition of restriction sites (XmaI and PstI) for subsequent

381   digestion. To form stable lines via the formation of complex arrays, the expression

382   construct The *Ppa-stdh-1*::RFP was digested with PstI and 5ng/µl of this, co-injected

383   into the germlines of young adult *P. pacificus* worms with the marker *Ppa-egl-*

384   *20*::Venus (10 ng/µl), and genomic carrier DNA (60ng/µl), also digested with PstI [38].

385   For the *Ppa-acs-19.1*::RFP construct, 10ng/µl of the construct cut with PstI, was

386   injected with the marker *Ppa-egl-20*::RFP (10ng/µl), and genomic carrier DNA (60ng/

387   µl) also cut with PstI*.* At least two independent lines were obtained from

388   microinjections for both transgenes.

389 **Transposon mutagenesis of bacteria**

390 To generate electro-competent cells of *N. lindaniclasticum* LE124 for electroporation,

391 *N. lindaniclasticum* LE124 cells were grown in LB overnight at 30° C. These

392 overnight cultures were diluted (1:10 vol/vol) and incubated for $\cong$ 6 h to reach early

393 log phase (optical density [OD] at 600nm of 0.3). The culture was centrifuged at 4° C,

394 10,000 rpm for 10 min before being washed once with ice-cold distillated water and

395 two times with ice-cold 10% glycerol. After the final washing step, cells were

396 centrifuged and the pellet re-suspended with $\cong$ 1 ml 10% glycerol before 50µl

397 aliquots were distributed to 1.5 ml Eppendorf tubes. The cells in glycerol were

398 electroporated with the EZ-Tn5 R6Ky*ori*/KAN-2>Tnp transposon (Epicentre, Madison

399 WI) using an Eppendorf Electroporator 2510 at 2.5 kV yielding around 5 ms. After

400 electroporation, the sample was immediately mixed with SOC (super optimal broth

401 with catabolite repression) medium and incubated at 30° C for two hrs, the culture

402 was then plated on LB agar medium supplemented with 50µg/ml of kanamycin.

403 **Bacterial transposon mutagenesis library preparation**

404 After two days incubation of the bacteria at 30° C, 10 colonies were randomly

405 selected, picked and a PCR carried out together with Sanger sequencing to confirm

406 the integration of the transposon into the *N. lindaniclasticum* LE124 genome using

407 the primers

408 KAN-2 FP-1 - F

409 5′-ACCTACAACAAAGCTCTCATCAACC-3′ and

410 R6KAN-2 RP-1 - R

411 5′-CTACCCTGTGGAACACCTACATCT-3′.

412

413 After successful confirmation of the bacterial transposon mutagenesis, around 4500

414 single mutant colonies were picked and inoculated to 96 well plates in 160µl LB

415 supplemented with 50 µg/ml of kanamycin. Overnight cultures of all mutants were

416 mixed with 160µl 50% glycerol and frozen at -80°C.

417 **Transposon mutant library screening using dietary sensors**

418   Transposon mutants were inoculated into 96 well plates in 180μl LB supplemented

419   with 50μg/ml of kanamycin. After overnight growth at 30° C, 20μl from the mutant

420   cultures were spotted to 24-well NGM plates. Bacterial mutant strains were incubated

421   for two days and eggs of *P. pacificus* RS3271 (*Ppa-stdh-1::*RFP*)* or *P. pacificus*

422   RS3379 (*Ppa-acs-19.1*::RFP) were bleached and filtered with Millipore 120.0μm

423   filters to reduce the amount of adult worm carcasses. Around 50-100 bleached eggs

424   were spotted to each well with mutant bacteria; *E. coli OP50* and *N. lindaniclasticum*

425   LE124 wild type strain were used as controls. Fluorescent worms were grown on the

426   bacterial strains until they became young adults. The *Ppa-stdh-1::*RFP line was

427   screened for decreased RFP expression while the *Ppa-acs-19.1*::RFP line was

428   screened for increased RFP expression. Initial positive results were re-screened at

429   least three times to confirm changes in gene expression.

430   **Analysis of Transposon Mutant Sequencing Data**

431   Raw reads were aligned against *N. lindaniclasticum* LE124 reference genome and

432   transposon sequence by the BWA aln and samse programs (version 0.7.12-r1039)[39].

433   The generated sam files were screened for read pairs where one read aligned to the

434   transposon sequence and the second read was unmapped. The location of the

435   affected gene was identified by realignment of the unmapped second read against

436   the *N. lindaniclasticum* LE124 reference with the help of blastn (version: 2.6.0)[40].

437   **Generation of CRISPR-induced mutants of *Ppa-metr-1* and *Ppa-mce-1***

438   We generated mutant alleles for *Ppa-metr-1* and *Ppa-mce-1* using the CRISPR/Cas9

439   technique following the protocol described previously (Witte et al, 2015). crRNAs

440   were synthesized by Integrated DNA Technologies and fused to tracrRNA (also

441   Integrated DNA Technologies) at 95° C for five min before the addition of the Cas9

442   endonuclease (New England Biolab). After a further five min incubation at RT, TE

443   buffer was added to a final concentration of 18.1μM for the sgRNA and 2.5μM for

444   Cas9. Around 20 young adults were injected; eggs from injected P0s were recovered

445   up to 16 hrs post injection. After hatching and two days of growth these F1 were

446   picked onto individual plates until they had also developed and laid eggs. The

447   genotype of the F1 animals was subsequently analyzed via Sanger sequencing and

448   mutations identified and isolated in homozygosity.

Phylogenetic Analysis

For two fatty acid metabolism related genes with differential expression between the bacterial diets, we retrieved homologs by BLASTP searches against WormBase (version: WS270) and pristionchus.org (version: TAU2011). Homologous protein sequences from *C. elegans* and *P. pacificus* were aligned by MUSCLE (version: 3.8.31) [41]) and maximum likelihood trees were generated with the help of the phangorn package in R (version: 3.5.3, parameters: model="LG", optNni=TRUE, optBf=TRUE, optInv=TRUE)[42]. To assess the robustness of the resulting trees, 100 bootstrap pseudoreplicates were calculated. For two *C. elegans* candidate genes involved in the Vitamin B12 pathway, one-to-one orthologs in *P. pacificus* could directly be retrieved from BLASTP searches against WormBase (version: WS270): *Ppa-metr-1* (PPA25255) and *Ppa-mce-1* (PPA39850). One-to-one orthology was confirmed by phylogenetic analysis.

Metabolite supplementation

Methylcobalamin (Vitamin B12 CAS Number 13422-55-4) and L-methionine (CAS Number 63-68-3) were purchased from Sigma and dissolved in water at the highest possible soluble concentrations to prepare stock solution. A methylcobalamin stock was prepared fresh before use in each experiment. Metabolite solutions were mixed with NGM agar at the required concentration just before pouring the 6 cm plates. Plates were allowed to dry at RT for two days and then spotted with *E. coli* OP50.

***Ppa-acs-19.1*::RFP gene expression screening on metabolite supplemented plates**

We used *Ppa-acs-19.1*::RFP transgenic animals to determine working concentrations of metabolite supplementations. Bleached *Ppa-acs-19.1*::RFP transgenic eggs were transferred to metabolite-supplemented plates, which were prepared as described above. *Ppa-acs-19.1*::RFP positive young adults were screened for differences in gene expression in comparison to control animals grown on a *E. coli* OP50 and *N. lindaniclasticum* LE124 diet without metabolite supplementation.

Imaging transgenic reporter lines

478   Eggs of transgenic reporter lines *Ppa-acs-19.1*::RFP and *Ppa-stdh-1*::RFP were

479   bleached and transferred to bacteria plates that were prepared as described. Three

480   ml of 2% agar was prepared and a drop (150μl) of 1 M sodium azide (NaN_3) was

481   added and mixed with agar to immobilize the worms. Around 200-μl agar was

482   dropped on microscope slide and young adult transgenic worms were placed on the

483   agar. Images of the worms were taken with 10X objective of ZEISS Imager Z1

484   equipped with the AxioCam camera using ZEN imaging software. The same

485   exposure time was applied to all images.

486   **Vitamin B12 (Methylcobalamin) supplementation assays**

487   Vitamin B12-supplemented plates were prepared as described above. *P. pacificus*,

488   *C. elegans*, *Rhabditophanes* sp. KR3021*, A. sudhausi* SB413, as well as *Ppa-metr-1*

489   (*tu1436, tu1436*) and *Ppa-mce-1* (*tu1433, tu1434* and *tu1435*) mutant animals were

490   grown on supplemented plates from egg to young adult stage and subsequently used

491   for i) predatory assays, ii) worm size measurements and iii) developmental assays.

492   For supplementation experiments with free-living *P. trichosuri,* J2 larvae were washed

493   five times with M9 medium and filtered with Millipore 20.0μm filters before being

494   soaked in PBS supplemented with 100μg/ml penicillin and ampicillin for one h to

495   avoid contamination. J2 larvae were washed a final time with PBS containing no

496   antibiotics and transferred to assay plates. For *S. carpocapsae,* J2 larvae were

497   washed with M9 medium and filtered with Millipore 20.0μm filters before transferring

498   to NGM plates supplemented with/without 500nM vitamin B12.

499   **Worm size measurement**

500   *P. pacificus*, *C. elegans*, *Rhabditophanes* sp., *P. trichosuri, A. sudhausi* and *S.*

501   *carpocapsae* synchronized young adults were transferred from assay plates to NGM

502   plates without bacteria. Bright field images of the worms were taken using 0.63x

503   objective of ZEISS SteREO Discovery V12 using the AxioCam camera. Images were

504   analyzed using the Wormsizer plug in for Image J/Fiji[36]. Wormsizer detects and

505   measures the volume of the worms.

Development rate assays

For development rate assays, *P. pacificus*, *C. elegans, Rhabditophanes sp.* and *A. sudhausi* were grown on OP50 at 20° C. Nematode eggs were bleached, washed with M9 several times and allowed to hatch in M9 medium for 20 hrs in the absence of food to cause J2 arrest. Once synchronized, J2 larvae were filtered through two Millipore 20.0μm filters and around 30-60 J2 animals were transferred to NGM plates (supplemented with/without 500nM vitamin B12) spotted in 50μl of the desired test bacterial strain. Nematodes were subsequently allowed to develop on test bacteria for the following time periods: *P. pacificus 57* hrs at 20° C, *C. elegans* and *Rhabditophanes sp.* 45 hrs at 20° C and *A. sudhausi* for 144 hrs at RT. Following this, worms were categorized into groups based on the development of the vulva and germ line using 0.63x objective of ZEISS SteREO Discovery V12 following previously established protocols[27].

Statistical analysis

Statistical calculations (mean, SEM, and t test) were performed by using R studio software. Pairwise t-tests with Benjamini-Hochberg multiple testing correction were applied when testing the effect of a single treatment or mutant against one single control sample. For tests across different groups (e.g. treatments, mutants, behaviors), Tukey-HSD test was applied. Significance is designated between two samples according to the following scale: 0 '***' 0.001 '**' 0.01 '*' 0.05 'n.s' 0.1 'n.s' 1.

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

N.A. and J.W.L. performed all behavioral experiments. W.R. performed the RNA-seq experiments, H.W., N.A. and J.W.L. generated dietary sensor lines and CRISPR-induced mutants. Bioinformatic analysis was performed by W-S.L. and C.R. All experiments were designed by N.A., C.R., J.W.L. and R.J.S.

DECLARATION OF INTERESTS

Authors declare no competing interests.

DATA AND MATERIAL AVAILABILITY

RNA-seq data has been deposited at the European Nucleotide Archive under the study accession PRJEB33410. All other data is available in the main text or the supplementary materials.

REFERENCES

1. Kamareddine, L., Robins, W. P., Berkey, C. D., Mekalanos, J. J. & Watnick, P. I. The Drosophila Immune Deficiency Pathway Modulates Enteroendocrine Function and Host Metabolism. *Cell Metabolism* **28,** 449-462.e5 (2018).

2. Thaiss, C. A., Zmora, N., Levy, M. & Elinav, E. The microbiome and innate immunity. *Nature* **535,** 65–74 (2016).

3. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **474,** 327–336 (2011).

4. Sommer, R. J., Carta, L. K., Kim, S. Y. & Sternberg, P. W. Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda: Neodiplogastridae). *Fundamental and Applied Nematology* **19,** 511–521 (1996).

5. Sommer, R. J. Integrative evolutionary biology and mechanistic approaches in comparative biology. In: *Pristionchus pacificus* - a nematode model for comparative and evolutionary biology (Ed.: R. J. Sommer). Brill, Leiden, pp. 19-41 (2015)

6. Wilecki, M., Lightfoot, J. W., Susoy, V. & Sommer, R. J. Predatory feeding behaviour in Pristionchus nematodes is dependent on phenotypic plasticity and induced by serotonin. *Journal of Experimental Biology* **218,** 1306–1313 (2015).

7. Ragsdale, E. J., Müller, M. R., Rödelsperger, C. & Sommer, R. J. XA developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell* **155,** 922 (2013).

8. Serobyan, V. *et al.* Chromatin remodelling and antisense-mediated up-regulation of the developmental switch gene *eud-1* control predatory feeding plasticity. *Nature Communications* **7,** (2016).

9. Sieriebriennikov, B. *et al.* A Developmental Switch Generating Phenotypic Plasticity Is Part of a Conserved Multi-gene Locus. *Cell Reports* **23,** 2835-2843.e4 (2018).

10. Lightfoot, J. W. *et al.* Small peptide–mediated self-recognition prevents cannibalism in predatory nematodes. *Science* **364,** 86–89 (2019).

11. Bento, G., Ogawa, A. & Sommer, R. J. Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature* **466,** 494–497 (2010).

12. Sanghvi, G. V. *et al.* Life history responses and gene expression profiles of the nematode *Pristionchus pacificus* cultured on *Cryptococcus* yeasts. *PLoS ONE* **11,** (2016).

13. Werner, M. S. *et al.* Environmental influence on *Pristionchus pacificus* mouth form through different culture methods. *Scientific Reports* **7,** (2017).

14. Kruuk, H. Surplus killing by carnivores. *Journal of Zoology* **166,** 233–244 (1972).

578 15. Nunn, G. L., Klem, D., Kimmel, T. & Merriman, T. Surplus killing and caching by

579 American kestrels (Falco sparverius). *Animal Behaviour* **24,** 759–763 (1976).

580 16. Mueller, D. L. & Hastings, B. C. A clarification of "surplus killing." *Animal*

581 *Behaviour* **25,** 1065 (1977).

582 17. Zimmermann, B., Sand, H., Wabakken, P., Liberg, O. & Andreassen, H. P.

583 Predator-dependent functional response in wolves: From food limitation to surplus

584 killing. *Journal of Animal Ecology* **84,** 102–112 (2015).

585 18. Reimchen, T. E. Some ecological and evolutionary aspects of bear-salmon

586 interactions in coastal British Columbia. *Canadian Journal of Zoology* **78,** 448–457

587 (2000).

588 19. Gaydos, J. K., Raverty, S., Baird, R. W. & Osborne, R. W. Suspected surplus

589 killing of harbor seal pups by killer whales. *Northwestern Naturalist* **86,** 150–154

590 (2005).

591 20. Veselý, L. *et al.* Effects of prey density, temperature and predator diversity on

592 nonconsumptive predator-driven mortality in a freshwater food web. *Scientific*

593 *Reports* **7,** (2017).

594 21. Maupin, J. L. Superfluous killing in spiders: a consequence of adaptation to food-

595 limited environments? *Behavioral Ecology* **12,** 569–576 (2001).

596 22. Lounibos, L. P., Makhni, S., Alto, B. W. & Kesavaraju, B. Surplus killing by

597 predatory larvae of *Corethrella appendiculata*: Prepupal timing and site-specific

598 attack on mosquito prey. *Journal of Insect Behavior* **21,** 47–54 (2008).

599 23. Trubl, P., Blackmore, V. & Chadwick Johnson, J. Wasteful Killing in Urban Black

600 Widows: Gluttony in Response to Food Abundance. *Ethology* **117,** 236–245 (2011).

601 24. Akduman, N., Rödelsperger, C. & Sommer, R. J. Culture-based analysis of

602 Pristionchus-associated microbiota from beetles and figs for studying nematode-

603 bacterial interactions. *PLoS ONE* **13,** (2018).

604 25. Saxena, A. *et al.* Genome sequence of *Novosphingobium lindaniclasticum*

605 LE124T, isolated from a hexachlorocyclohexane dumpsite. *Genome Announcements*

606 **1,** (2013).

607 26. Yamada, K. in (eds. Sigel, A., Sigel, H. & Sigel, R. K. O.) 295–320 (Springer

608 Netherlands, 2013). doi:10.1007/978-94-007-7500-8_9

609 27. Watson, E. *et al.* Interspecies systems biology uncovers metabolites affecting *C.*

610 *elegans* gene expression and life history traits. *Cell* **156,** 759–770 (2014).

611 28. Kumar, N. in *Handbook of Clinical Neurology* **120,** 915–926 (Elsevier B.V., 2014).

612 29. Prabh, N. *et al.* Deep taxon sampling reveals the evolutionary dynamics of novel

613 gene families in *Pristionchus* nematodes. *Genome Research* **28,** 1664–1674 (2018).

614 30. Bito, T., Matsunaga, Y., Yabuta, Y., Kawano, T. & Watanabe, F. Vitamin B 12
615 deficiency in *Caenorhabditis elegans* results in loss of fertility, extended life cycle,
616 and reduced lifespan. *FEBS Open Bio* **3,** 112–117 (2013).
617 31. Na, H., Ponomarova, O., Giese, G. E. & Walhout, A. J. M. *C. elegans* MRP-5
618 Exports Vitamin B12 from Mother to Offspring to Support Embryonic Development.
619 *Cell Reports* **22,** 3126–3133 (2018).
620 32. Bumbarger, D. J., Riebesell, M., Rödelsperger, C. & Sommer, R. J. System-wide
621 rewiring underlies behavioral differences in predatory and bacterial-feeding
622 nematodes. *Cell* **152,** 109–119 (2013).
623 33. Okumura, M., Wilecki, M. & Sommer, R. J. Serotonin drives predatory feeding
624 behavior via synchronous feeding rhythms in the nematode *Pristionchus pacificus*.
625 *G3: Genes, Genomes, Genetics* **7,** 3745–3755 (2017).
626 34. Hong, R. L. *et al.* Evolution of neuronal anatomy and circuitry in two highly
627 divergent nematode species. *eLife*, *8*, e47155 (2019). doi:10.7554/eLife.47155
628 35. Grant, W. N. *et al. Parastrongyloides trichosuri*, a nematode parasite of mammals
629 that is uniquely suited to genetic analysis. *International Journal for Parasitology* **36,**
630 453–466 (2006).
631 36. Moore, B. T., Jordan, J. M. & Baugh, L. R. WormSizer: High-throughput Analysis
632 of Nematode Size and Shape. *PLoS ONE* **8,** (2013).
633 37. Trapnell, C. *et al.* Differential gene and transcript expression analysis of RNA-seq
634 experiments with TopHat and Cufflinks. *Nature Protocols* **7,** 562–578 (2012).
635 38. Schlager, B., Wang, X., Braach, G. & Sommer, R. J. Molecular cloning of a
636 dominant roller mutant and establishment of DNA-mediated transformation in the
637 nematode *Pristionchus pacificus*. *Genesis* **47,** 300–304 (2009).
638 39. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler
639 transform. *Bioinformatics* **26,** 589–595 (2010).
640 40. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local
641 alignment search tool. *Journal of Molecular Biology* **215,** 403–410 (1990).
642 41. Edgar, R. C. MUSCLE: Multiple sequence alignment with high accuracy and high
643 throughput. *Nucleic Acids Research* **32,** 1792–1797 (2004).
644 42. Schliep, K. P. phangorn: Phylogenetic analysis in R. *Bioinformatics* **27,** 592–593
645 (2011).

FIGURE LEGENDS

647 **Figure 1. Bacterial diet modulates killing behavior in *P. pacificus***
648 (**A**) Eurystomatous (Eu) and stenostomatous (St) mouth forms. Eu worms are
649 capable of predation and have a wide mouth with two teeth, while St worms feed on
650 bacteria and have a narrower mouth with one tooth. (**B**) A predatory *P. pacificus*
651 adult biting a *C. elegans* larvae. (**C**) Corpse assay of *P. pacificus* predators fed upon
652 *C. elegans* larvae following growth on a variety of ecologically associated bacteria;
653 five predators are fed prey for two hours for each assay. *N* = 5 replicates for each
654 assay. (**D**) Bite assay after growth on either an *E. coli* OP50 or *Novosphingobium*
655 L76 diet to assess the effect on *P. pacificus* surplus-killing behavior. Numbers of
656 bites, successful bites and feeding was quantified during a 10 min interval while fed
657 upon *C. elegans* larvae. (**E**) A corpse assay of *P. pacificus* fed with *E. coli* OP50,
658 *Novosphingobium* L76 or of *E. coli* OP50 with *Novosphingobium* L76 supernatant.
659 N=10 replicates for each assay for (D) and (E).

660 **Figure 2. Bacterial diet influences gene expression in *P. pacificus***
661 (**A**) RNA-seq analysis of *P. pacificus* in response to a diet of *Novosphingobium* L76
662 compared to *E. coli* OP50. The pathways with most significant enrichment (FDR-
663 corrected $P<10^{-5}$) in downregulated and (**B**) upregulated genes are shown. (**C**) The
664 dietary sensor *Ppa-acs-19.1*::RFP is highly expressed in ventral gland, hypodermal
665 and intestinal cells following an *E. coli* OP50 diet, while a *Novosphingobium* L76 diet
666 induces expression only in ventral gland cells. The co-injection marker *Ppa-egl-*
667 *20*::RFP is expressed in the tail. (**D**) *Ppa-stdh-1*::RFP is expressed in the intestinal
668 and hypodermal cells with expression strongly upregulated on *Novosphingobium* L76
669 diet compared to an *E. coli* OP50 diet. (**E**) Expression of *Ppa-acs-19.1*::RFP dietary
670 sensor after feeding on *N. lin.* LE124 transposon mutants with mutations in vitamin
671 B12 (*N. lin.* LE124 *CbiQ::Tn5)*, purine (*N. lin.* LE124 *PurH::Tn5*), pyrimidine
672 biosynthesis (*N. lin.* LE124 *PryD::Tn5*) and nitrogen metabolism (*N. lin.* LE124
673 *GlnD::Tn5*). Mutants increase the expression of the dietary sensor in comparison to a
674 *N. lin.* LE124 wild-type diet. (**F**) Corpse assay of *P. pacificus* after feeding on various
675 *N. lin.* LE124 mutants. There is decreased killing efficiency compared to a *N. lin.*
676 LE124 wild type diet. N=10 replicates for each assay.

677   **Figure 3. Vitamin B12 containing diet regulates surplus killing behavior and**

678   **development.**

679   (**A**) Corpse assays showing effects of vitamin B12 supplementation on *P. pacificus*

680   predation efficiency with *P. pacificus* fed on either *E. coli* OP50*, N. lin.* LE124, *N. lin.*

681   LE124 *CbiQ::Tn5*, 500nM vitamin B12 supplemented *E. coli* OP50 or 500nM vitamin

682   B12 supplemented *N. lin.* LE124 *CbiQ::Tn5* prior to assays*. (***B***)* Bite assays showing

683   effects of vitamin B12 supplementation on *P. pacificus* killing behavior with *P.*

684   *pacificus* fed on either *E. coli* OP50*, N. lin.* LE124, *N. lin.* LE124 *CbiQ::Tn5* ,500nM

685   vitamin B12 supplemented *E. coli* OP50 or 500nM vitamin B12 supplemented *N. lin.*

686   LE124 *CbiQ::Tn5* prior to assays. (**C**) Developmental staging of *C. elegans* and *P.*

687   *pacificus* showing percentage of L3, early L4, mid L4, late L4 and young adults on

688   plates after feeding with *E. coli* OP50, *Commamonas* DA18877 and *N. lin.* LE124 for

689   either 45 hours (*C. elegans*) or 56 hours (*P. pacificus).* (**D**) Corpse assays of *P.*

690   *pacificus* fed with *E. coli* OP50, *Commamonas* DA18877 and *N. lin.* LE124. N=10

691   replicates for each assay in figure.

692   **Figure 4. Vitamin B12 influence on development is conserved in various**

693   **nematodes.**

694   (**A**) Corpse assays of *P. pacificus* wild-type (PS312) and mutant animals defective in

695   vitamin B12-dependent pathways *Ppa-metr-1* and *Ppa-mce-1* fed with *E. coli* OP50

696   supplemented with/without 500nM vitamin B12. (**B**) Corpse assays of PS312 and

697   *Ppa-metr-1* fed with *E. coli* OP50 supplemented with/without 10mM methionine.

698   N=10 replicates for each assay. (**C**) and (**D**) Comparative volume measurement of *C.*

699   *elegans, P. pacificus, Parastrongyloides trichosuri, Rhabditophanes sp.,*

700   *Steinernema carpocapsae and Allodiplogaster sudhausi* after growing on bacterial

701   plates supplemented with vitamin B12 versus non-supplemented plates. N=60 for

702   each assay.

SUPPLEMENTARY FIGURE LEGENDS

704 **Figure S1. Bacterial diet affects predatory behavior in *P. pacificus.***

705 (**A**) Mouth form ratio of *P. pacificus* PS312 after feeding with 25 different bacteria

706 strains. Bacterial diet fails to influence mouth-form ratio. N=3 replicates for each

707 assay. (**B**) Pharyngeal pumping behavior of *P. pacificus* PS312 on 25 different

708 bacterial diets. N=20 replicates for each assay.

709 (**C**) Corpse assay illustrating affect of bacterial diet switching from *E. coli* OP50 to

710 *Novosphingobium* L76 at particular *P. pacificus* development stages. Corpse assays

711 were performed with young adults suggesting feeding with *Novosphingobium* L76 at

712 diverse developmental stages modify killing behavior. (**D**) Corpse assays of *P.*

713 *pacificus* previously fed with a mixture of *Novosphingobium* L76 and *E. coli* OP50 at

714 1/10, 1/100 and 1/1000 concentrations. Low concentrations of *Novosphingobium* L76

715 in the diet is sufficient to influence killing behavior. Bacteria were spotted to NGM

716 without peptone to prevent bacterial growth. N=10 replicates for each assay. **(E)**

717 Corpse assays of *P. pacificus* previously fed on either Novosphingobium L76 or

718 Novosphingobium LE124. The increased killing behaviors are observed in both

719 strains of Novosphingobium. N=10 replicates for each assay.

720 **Figure S2. Mutations in multiple pathways affect dietary sensor expression and**

721 **predatory behavior.**

722 **(A)** A phylogenetic analysis of acs-19 and let-767 homologs indicates that individual

723 members of the Acyl CoA synthtase family and **(B)** the steroid dehydrogenase family

724 (panel B) have undergone lineage specific duplications. Nodes with bootstrap

725 support ≥ 90/100 are labeled with stars and arrows mark *P. pacificus* genes that were

726 used as dietary sensors.

727 (**C**) Images of *Ppa-acs-19.1*::RFP dietary sensor showing purine (*N. lin.* LE124

728 *PurA::Tn5, N. lin.* LE124 *PurD::Tn5, N. lin.* LE124 *PurE::Tn5, N. lin.* LE124

729 *GuaB::Tn5* and *N. lin.* LE124 *PurM::Tn5)* and pyrimidine biosynthesis (*N. lin.* LE124

730 *PryE::Tn5*) mutants increase the expression of the dietary sensor in comparison to

731 *N. lin.* LE124 wild-type diet. (**D**) Corpse assays of *P. pacificus* fed with *N. lin.* LE124

732 mutants from vitamin B12 (green), purine (white), pyrimidine biosynthesis (grey) and

733 nitrogen metabolism (dark grey) all decreasing killing efficiency in comparison to *N.*

734 *lin.* LE124 wild-type diet. N=10 replicates for each assay. (**E**) Bite assays of *P.*

735 *pacificus* previously fed on *E. coli* OP50*, N. lin.* LE124 and *N. lin.* LE124 mutants

736   from vitamin B12 (green), purine (white), pyrimidine biosynthesis (grey) and nitrogen

737   metabolism (dark grey) modulating killing efficiency. Ten replicates for each assay.

738   **Figure S3. Vitamin B12 regulates fatty acid gene expression and development.**

739   (**A**) *Ppa-acs-19.1* transgenic worms were grown on NGM plates supplemented with

740   various concentrations of vitamin B12. NGM plates without vitamin B12 spotted with

741   *E. coli* OP50 and *N. lin.* LE124 were used as controls. Images of transgenic animals

742   were taken to determine the most efficient vitamin B12 concentration. Vitamin B12

743   Supplemented *E. coli* OP50 phenocopies *N. lin.* LE124 effect on *Ppa-acs-19.1*

744   expression.

745   (**B**) *Ppa-acs-19.1* transgenic worms were added to NGM plates with *N. lin.* LE124

746   transposon mutants and with/without supplementation with 500 nM vitamin B12. *E.*

747   *coli* OP50 and *N. lin.* LE124 were as controls. Vitamin B12 supplementation rescued

748   *Ppa-acs-19.1* expression on *N. lin.* LE124 *CbiQ::Tn5* mutant (blue highlighted box).

749   **Figure S4. Vitamin B12 dependent metabolic pathways.**

750   **(A)** Network of the main two vitamin B12-dependent pathways. *P.pacificus*

751   Orthologous of genes labeled in green were mutated with CRISPR/Cas9.

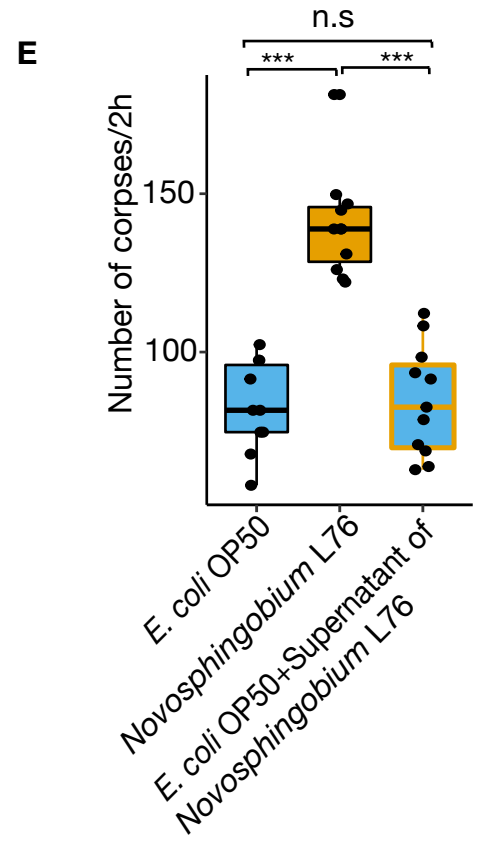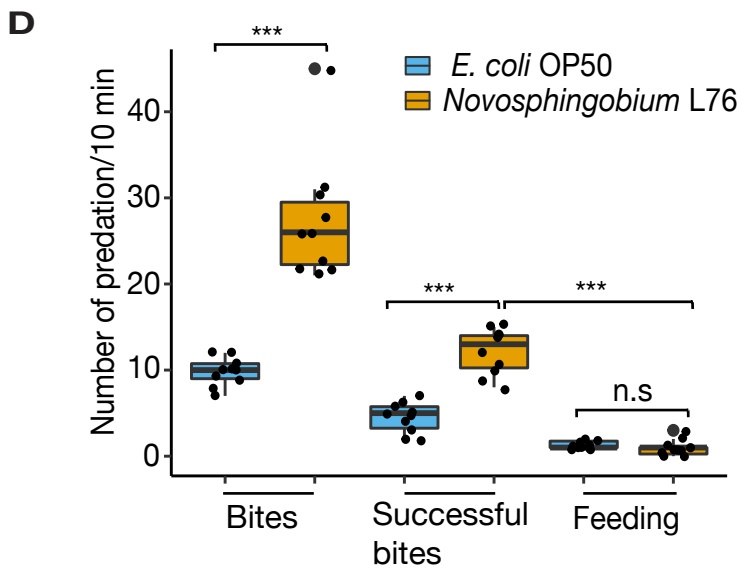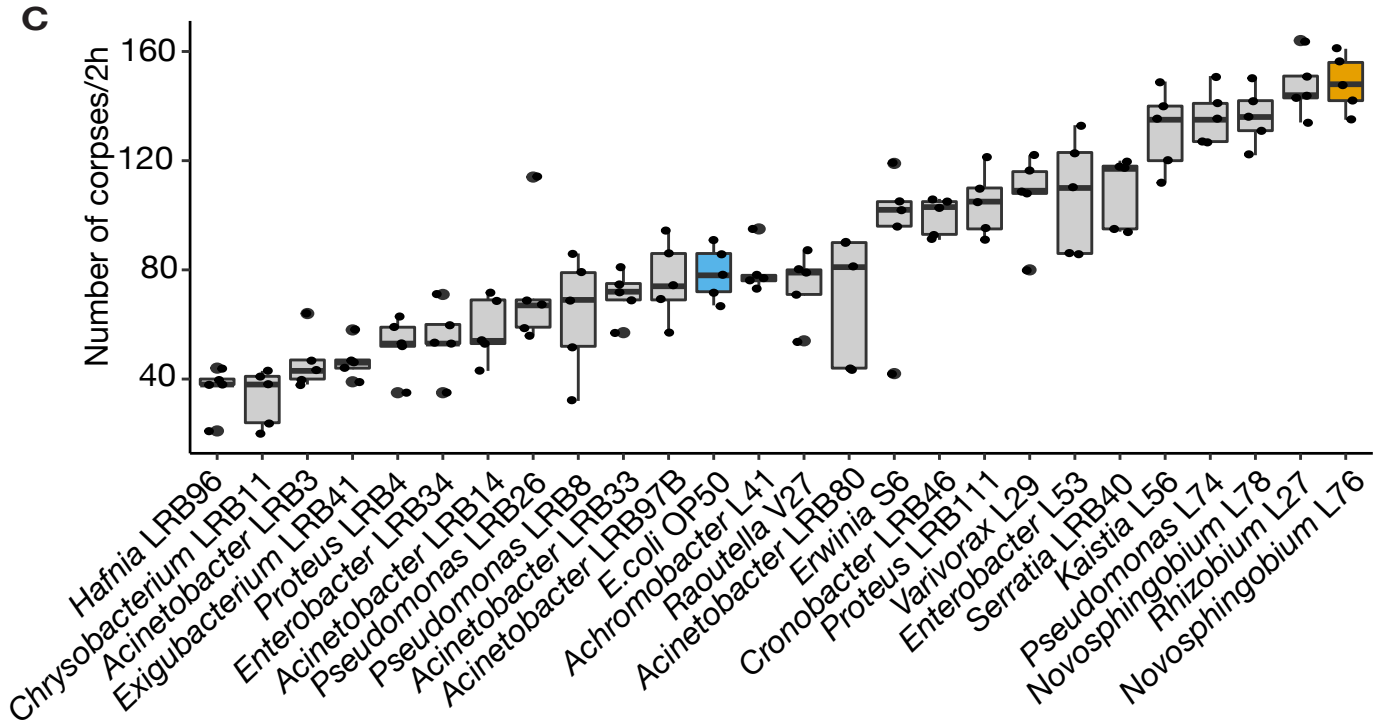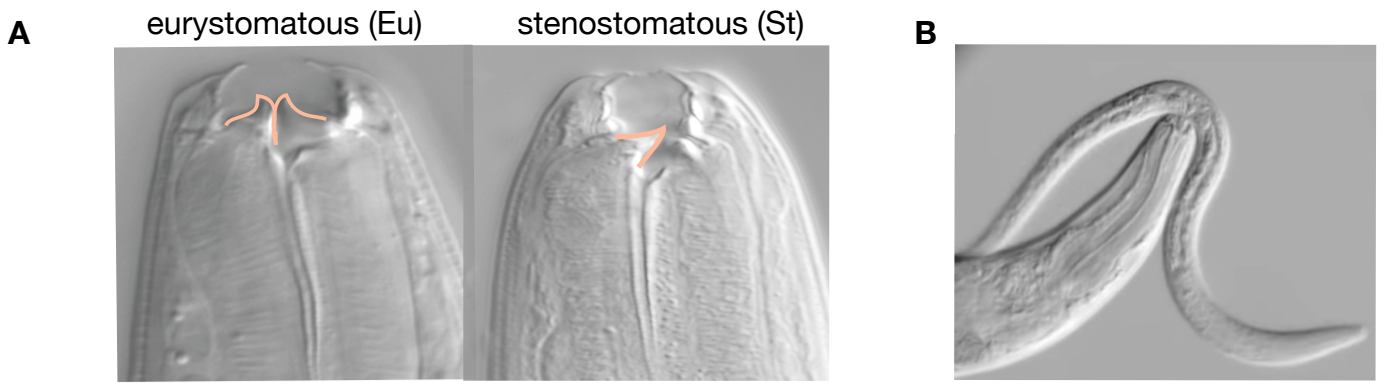752   Orthologous of red-labeled *acs-19* used as dietary sensor.

753   **(B)** One-to-one orthologs could be identified for *metr-1* **(C)** and *mce-1*. Nodes with

754   bootstrap support ≥ 90/100 are labeled with stars and arrows mark *P. pacificus*

755   genes that were used for functional studies. **(D)** Mutations were induced in both *Ppa-*

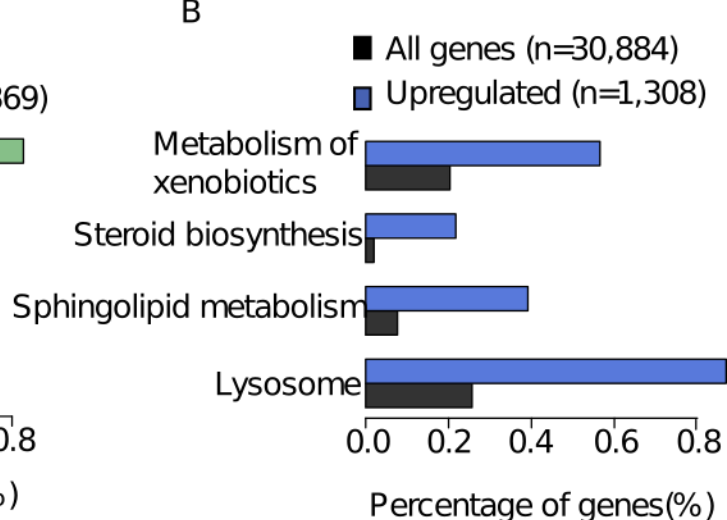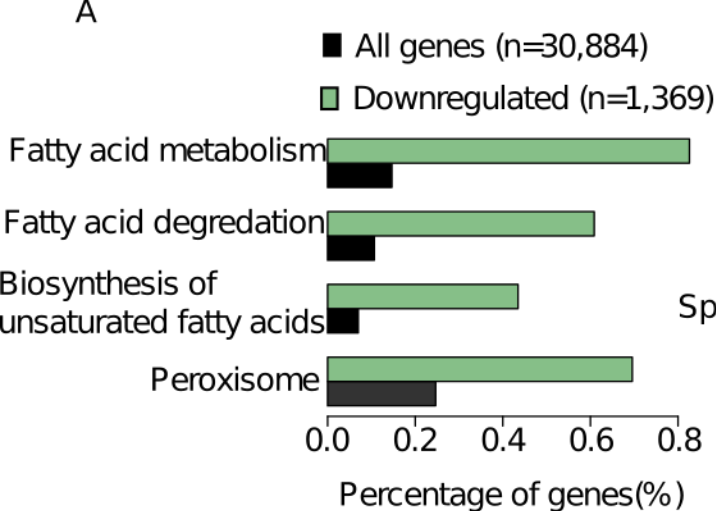756   *metr-1* and *Ppa-mce-1* using CRISPR/Cas9 with the

757   target locations indicated in both genes (scissors). Mutations induced via

758   CRIPSR/Cas9 are also shown. (**E**) and (**F**) Developmental staging of

759   *Rhabditophanes sp*. and *A. sudhausi* on *E. coli* OP50 NGM plates supplemented

760   with/without vitamin B12. The development of *Rhabditophanes sp*. and *A. sudhausi*

761   was accelerated with vitamin B12 supplementation. N=10 replicates for each assay.

SUPPLEMENTARY TABLE LEGENDS
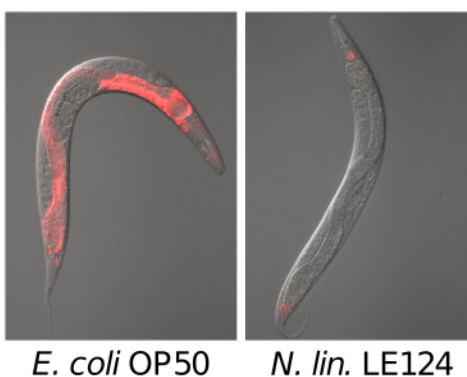
762 **SUPPLEMENTARY TABLE LEGENDS**

763 **Table S1.** List of strains and other resources that were used in this study.

764 **Table S2.** List of differentially expressed genes between *P. pacificus* grown on *E.coli*
765 OP50 and *Novosphingobium* L76 . List includes *P. pacificus* gene identifiers, the
766 associated expression fold changes, FDR corrected P-values and where appropriate
767 the identified *C. elegans* orthologous genes can be found in a separate excel file.
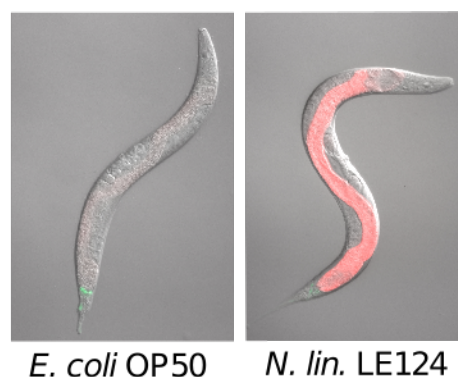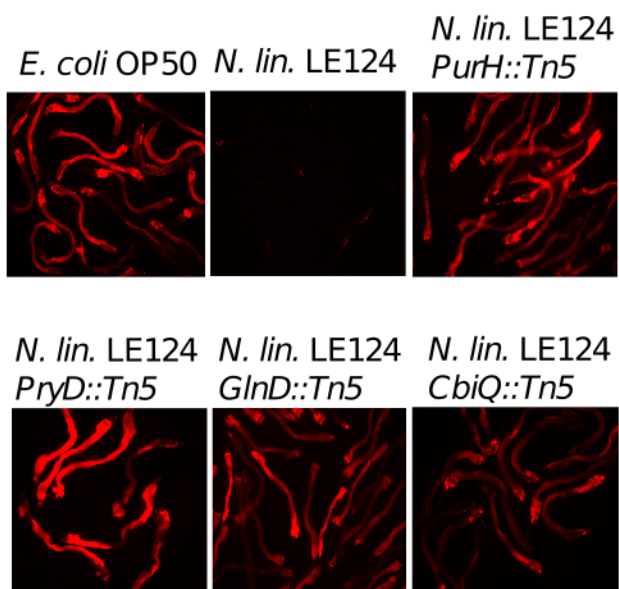
28

A eurystomatous (Eu)   stenostomatous (St)

B

C

Number of corpses/2h

Hafnia LRB96
Chrysobacterium LRB11
Acinetobacter LRB3
Exigubacterium LRB41
Proteus LRB4
Enterobacter LRB34
Acinetobacter LRB14
Pseudomonas LRB26
Pseudomonas LRB8
Acinetobacter LRB33
Acinetobacter LRB97B
E.coli OP50
Achromobacter L41
Raoutella V27
Acinetobacter LRB80
Erwinia S6
Cronobacter LRB46
Proteus LRB111
Varivorax L29
Enterobacter L53
Serratia LRB40
Kaistia L56
Pseudomonas L74
Novosphingobium L78
Rhizobium L27
Novosphingobium L76

D

Number of predation/10 min

E. coli OP50
Novosphingobium L76

Bites   Successful bites   Feeding

***   ***   ***   n.s

E

Number of corpses/2h

E. coli OP50
Novosphingobium L76
E. coli OP50+Supernatant of *Novosphingobium* L76

n.s   ***   ***

A All genes (n=30,884) / Downregulated (n=1,369)

Fatty acid metabolism
Fatty acid degradation
Biosynthesis of unsaturated fatty acids
Peroxisome

Percentage of genes(%)

B All genes (n=30,884) / Upregulated (n=1,308)

Metabolism of xenobiotics
Steroid biosynthesis
Sphingolipid metabolism
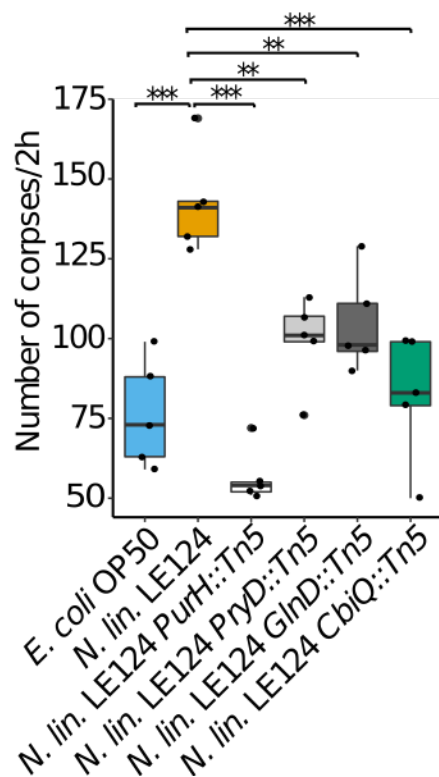Lysosome

Percentage of genes(%)

C *Ppa-acs-19.1*::RFP expression

E. coli OP50   *N. lin.* LE124

D *Ppa-stdh-1*::RFP expression

E. coli OP50   *N. lin.* LE124

E *Ppa-acs-19.1*::RFP expression

E. coli OP50   *N. lin.* LE124   *N. lin.* LE124 *PurH::Tn5*

N. lin. LE124 *PryD::Tn5*   *N. lin.* LE124 *GlnD::Tn5*   *N. lin.* LE124 *CbiQ::Tn5*

F

Number of corpses/2h

E. coli OP50
N. lin. LE124
N. lin. LE124 *PurH::Tn5*
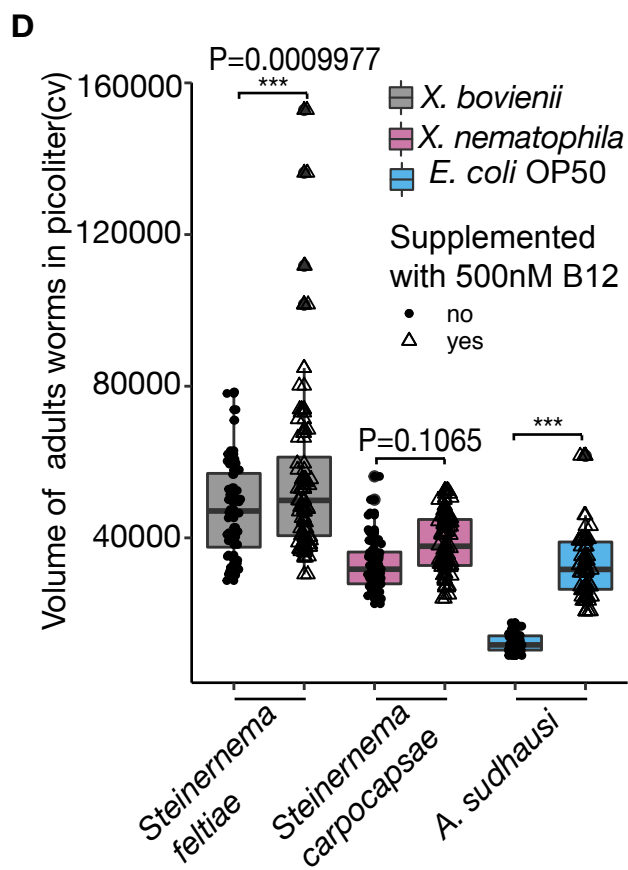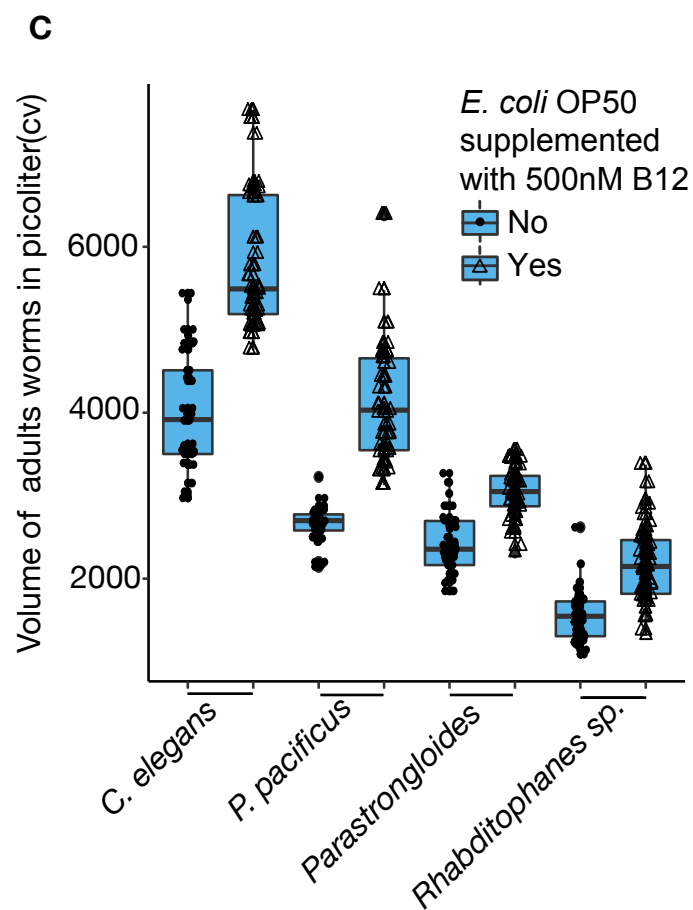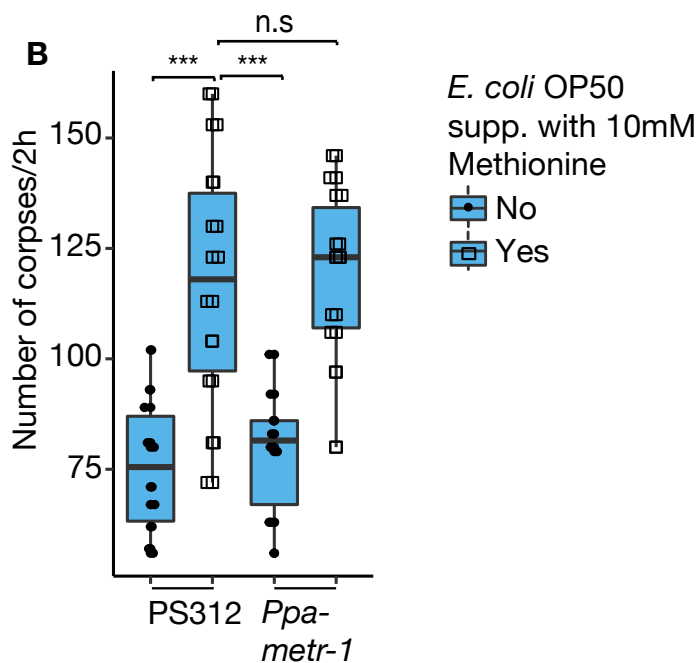*N. lin.* LE124 *PryD::Tn5*
N. lin. LE124 *GlnD::Tn5*
N. lin. LE124 *CbiQ::Tn5*

A

Percentage of Eu mouth form

Hafnia LRB96, *Chrysobacterium* LRB11, *Acinetobacter* LRB3, *Exigubacterium* LRB41, *Proteus* LRB4, *Enterobacter* LRB34, *Acinetobacter* LRB14, *Pseudomonas* LRB26, *Pseudomonas* LRB8, *Acinetobacter* LRB33, *Acinetobacter* LRB97B, *E. coli* OP50, *Achromobacter* L41, *Raoutella* V27, *Acinetobacter* LRB80, *Erwinia* S6, *Cronobacter* LRB46, *Proteus* LRB111, *Varivorax* L29, *Enterobacter* L53, *Serratia* LRB40, *Kaistia* L56, *Pseudomonas* L74, *Novosphingobium* L78, *Rhizobium* L27, *Novosphingobium* L76

B

Number of Pumping per 15 sec

Hafnia LRB96, *Chrysobacterium* LRB11, *Acinetobacter* LRB3, *Exigubacterium* LRB41, *Proteus* LRB4, *Enterobacter* LRB34, *Acinetobacter* LRB14, *Pseudomonas* LRB26, *Pseudomonas* LRB8, *Acinetobacter* LRB33, *Acinetobacter* LRB97B, *E. coli* OP50, *Achromobacter* L41, *Raoutella* V27, *Acinetobacter* LRB80, *Erwinia* S6, *Cronobacter* LRB46, *Proteus* LRB111, *Varivorax* L29, *Enterobacter* L53, *Serratia* LRB40, *Kaistia* L56, *Pseudomonas* L74, *Novosphingobium* L78, *Rhizobium* L27, *Novosphingobium* L76

C

Number of corpses/2h

E. coli OP50, *Novosphingobium* L76, L2, L3, L4

Bacterial diet switch stage

***, n.s, n.s, *, *

D

Number of corpses/2h

E. coli OP50, *Novosphingobium* L76, 1/10, 1/100, 1/1000

***, n.s, n.s, n.s

E

Number of corpses/2h

E. coli OP50, *Novosphingobium* L76, *N. lin.* LE124
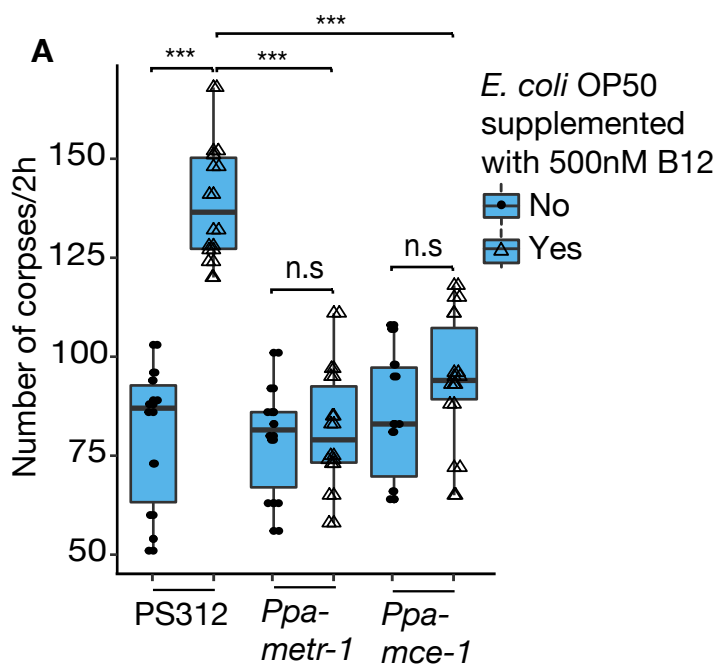
***, ***, n.s

A Acyl CoA synthetase family

B Steroid dehydrogenase family

C *Ppa-acs-19.1*::RFP expression

D

E

A

*Ppa-acs-19.1::*RFP expression *E. coli* OP50 diet

-B12 control	+50nM B12	+100nM B12	+250nM B12	+500nM B12	+1μM B12	LE124T control

B

	E. coli OP50	*Ppa-acs-19.1::*RFP expression on *Novosphingobium* LE124T										
		PurA	*PurD*	*PurE*	*PurH*	*PurM*	*GuaB*	*PyrD*	*PyrE*	*GlnD*	*CbiQ*	wt

A

Methylate DNA, RNA, proteins, membrane phospholipids, neurotransmitters

R → R-CH3

SAM ← Methionine
metr-1
B12

SAM → SAH ← Homocysteine
B6
Cystathionine
Cysteine ←
Cystathionine
α-ketobutyrate

Valine
Isoleucine → Propionyl-CoA ← Propionic acid
acs-19

Propionyl-CoA

D-methylmalonyl-CoA
mce-1
L-methylmalonyl-CoA
mmcm-1 ← B12
Succinyl-CoA

TCA cycle

B

1.0

cel_METR-1
*
cbr_METR-1

mic_METR-1_MicoRS5524-mkr-S6-1.63-mRNA-1
*
pex_METR-1 exspectatus-mkr-S_432-0.41-mRNA-3
*
ppa_METR-1 PPA25255 ←
*
para_METR-1 Parapristionchus-sn_msk-S_63-1.23-mRNA-1

sra_METR-1SRAE_2000434600

C

0.1

cel_MCE-1
*
cbr_MCE-1
*
mic_MCE-1_MicoRS5524-ag_msk-S5-8.74-mRNA-1

para_MCE-1_Parapristionchus-mkr-S_14-3.15-mRNA-1
*
ppa_PPA39850 ←
*
pex_MCE-1_exspectatus-ag_msk-S_890-0.28-mRNA-1

sra_MCE-1_SRAE_2000013200

D

Ppa wild type   ATGCAGGAGCCGATATAATCGGAACGAATACTTTCTCGGGAACGACCATTGCACAGGTAGAGGAAGATGA
Ppa-metr-1   ATGCAGGAGCCGATATAATCG---------------------------------ACAGGTAGAGGAAGATGA

Ppa-metr-1

11716000   11718000   11720000   11722000   11724000   11726000
ChrII

Ppa wild type   GGAGTGGCGATG- - - -GCCACATGGTTC
Ppa-mce-1   GGAATGCCGATGGCAAGCCACATGGTTC

Ppa-mce-1

9748000   9750000   9752000   9754000   9756000   9758000
ChrV

E

Rhabditophanes sp.

Percent total animals
100%
75%
50%
25%
0%

Adult
late L4
middle L4
early L4
L3

E. coli OP50
E. coli OP50 +500nM B12

F

Allodiplogaster sudhausi

Percent total animals
100%
75%
50%
25%
0%

Adult
L4
L3
L2

E. coli OP50
E. coli OP50 +500nM B12

Supplementary Table 1

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial strains		
E.coli OP50	Caenorhabditis Genetics Center (CGC)	RRID:WB-STRAIN:OP50
Comamonas aq. DA1877	Caenorhabditis Genetics Center (CGC)	N/A
Novosphingobium lindaniclasticum LE124	German collection of microorganisms and cell culture GmbH (DSMZ)	N/A
Hafnia LRB96	Akduman et al., 2018	N/A
Chrysobacterium LRB11	Akduman et al., 2018	N/A
Acinetobacter LRB3	Akduman et al., 2018	N/A
Exiguobacterium LRB41	Akduman et al., 2018	N/A
Proteus LRB4	Akduman et al., 2018	N/A
Enterobacter LRB34	Akduman et al., 2018	N/A
Acinetobacter LRB14	Akduman et al., 2018	N/A
Pseudomonas LRB26	Akduman et al., 2018	N/A
Pseudomonas LRB8	Akduman et al., 2018	N/A
Acinetobacter LRB33	Akduman et al., 2018	N/A
Acinetobacter LRB97B	Akduman et al., 2018	N/A
Achromobacter L41	Akduman et al., 2018	N/A
Raoultella V27	Akduman et al., 2018	N/A
Acinetobacter LRB80	Akduman et al., 2018	N/A
Erwinia S6	Akduman et al., 2018	N/A
Cronobacter LRB46	Akduman et al., 2018	N/A
Proteus LRB111	Akduman et al., 2018	N/A
Variovorax L29	Akduman et al., 2018	N/A
Enterobacter L53	Akduman et al., 2018	N/A
Serratia LRB40	Akduman et al., 2018	N/A
Kaistia L56	Akduman et al., 2018	N/A
Pseudomonas L74	Akduman et al., 2018	N/A
Novosphingobium L78	Akduman et al., 2018	N/A
Rhizobium L27	Akduman et al., 2018	N/A
Novosphingobium L76	Akduman et al., 2018	N/A
Novosphingobium lindaniclasticum LE124 *PurA::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PurD::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PurE::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PurH::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PurM::Tn5*	This study	N/A

Novosphingobium lindaniclasticum LE124 *GuaB::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PryD::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *PryE::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *GlnD::Tn5*	This study	N/A
Novosphingobium lindaniclasticum LE124 *CbiQ::Tn5*	This study	N/A
Chemicals, Peptides, and Recombinant Proteins		
Alt-R CRISPR-Cas9 tracrRNA	Integrated DNA Technologies	Cat#1072534
EnGen Cas9 NLS, *S. pyogenes*	New England Biolabs	Cat#M0646M
Methylcobalamin	Sigma-Aldrich	CAS Number 63-68-3
L-Methionine	Sigma-Aldrich	Lot#SLBZ1683
Kanamycin	Sigma-Aldrich	CAS Number 70560-51-9
FastDigest PstI	Thermofisher Scientfic	Cat# FD0615
FastDigest BamHI	Thermofisher Scientfic	Cat# FD0054
Gibson Assembly® Cloning Kit	New England Biolabs	Cat# E5510S
Experimental Models: Organisms/Strains		
Pristionchus pacificus: strain PS312	Stock of Dep. IV, MPI Developmental Biology Tuebingen	N/A
C. elegans; strain N2 Bristol	Caenorhabditis Genetics Center (CGC)	N/A
Rhabditophanes sp; strain KR3021	Stock of Dep. IV, MPI Developmental Biology Tuebingen	N/A
Parastrongyloides *trichosuri*	Stock of Dep. IV, MPI Developmental Biology Tuebingen	N/A
Allodiplogaster sudhausi : strain SB413	Stock of Dep. IV, MPI Developmental Biology Tuebingen	N/A
Steinernema carpocapsae	R. Ehlers	N/A
P. pacificus strain RS3271 (*Ppa-stdh-1::*RFP + *Ppa*-egl-20::Venus)	This study	N/A
P. pacificus strain RS3379	This study	N/A

(Ppa-*acs-19.1*::RFP + *Ppa*-egl-20::RFP)		
Pristionchus pacificus: strain RS3653: *mce-1* (tu1433)	This study	N/A
Pristionchus pacificus: strain RS3654: *mce-1* (tu1434)	This study	N/A
Pristionchus pacificus: strain RS3655: *mce-1* (tu1435)	This study	N/A
Pristionchus pacificus: strain RS3656: *met-1*(tu1436)	This study	N/A
Pristionchus pacificus: strain RS3657: *met-1*(tu1437)	This study	N/A
Oligonucleotides		
Ppa-stdh-1 - F: 5'-GCCAAGCTTGCATGCCTGCACATGCTATGGAGCGTAGC-3'	This study	N/A
Ppa-stdh-1 - R: 5'-CTGAAAAAAAAAACCCAAGCTTGGGTCCCGAAGACGACGTTGTAGAC-3';	This study	N/A
Ppa-acs-19.1 -F 5'-GGATCCCGTCGACCTGCAGGCATG-3	This study	N/A
Ppa-acs-19.1 -R 5'-ATGAGCGAGCTGATCAAG-3	This study	N/A
TurboRFP-F 5'-TGCATGCCTGCAGGTCGACGGGATCCGCCATCACTATGCATTGCTG-3'	This study	N/A
TurboRFP-R 5'-TCCTTGATCAGCTCGCTCATCTGAACCAGCAAGGGCGATAG-3	This study	N/A
KAN-2 FP-1 Forward Primer 5′-ACCTACAACAAAGCTCTCATCAACC-3′	Epicentre, Madison WI	Cat#TSM08KR
R6KAN-2 RP-1 Reverse Primer 5′-CTACCCTGTGGAACACCTACATCT-3′	Epicentre, Madison WI	Cat#TSM08KR
sgRNA target sequence: exon 2 of mce-1: CCATGTGGCCATCGCCACTC	This study	N/A
sgRNA target sequence: exon 11 of metr-1: AAAATGTATCTGGATGCAGG	This study	N/A
Recombinant DNA		
Plasmid: pUC19-*egl-20p::TurboRFP::rpl-23utr*	Schlager et al., 2009	N/A
Plasmid: pUC19-*egl-20p::Venus::rpl-23utr*	Okumura et al., 2017	N/A
Plasmid: pUC19-*acs-*	This study	N/A

19.1p::TurboRFP::rpl-23utr		
Plasmid: pUC19-*stdh-1::TurboRFP::rpl-23utr*	This study	N/A
EZ-Tn*5* R6Ky*ori*/KAN-2>Tnp transposon	Epicentre, Madison WI	Cat#TSM08KR
Software and Algorithms		
FIJI	Schindelin et al., 2012	N/A
R	http://www.r-project.org/	N/A
TopHat (version:2.0.14)	Trapnell et al. 2012	N/A
Cuffdiff (version: 2.2.1)	Trapnell et al. 2012	N/A
Other		N/A
Total RNA was extracted using Direct-zol RNA Kits	Zymo Research	Cat#R2051
Truseq RNA library prep kit was used to prepare RNA libraries	Illumina Company	Cat#RS-122-2001

4. Discussion

For my PhD study, I have first isolated and characterized bacterial strains from *Pristionchus*-associated environments, scarab beetles, soil and figs using culture dependent methods, where I used several general media for bacterial isolation (103). I isolated bacteria from samples that showed the presence of *Pristionchus* nematodes to obtain ecologically relevant results of microbial-animal interactions. However, using culture-dependent analysis, I could only isolate a small percentage of the total bacterial community. Nevertheless, these cultivable strains form a rich resource to study how *Pristionchus* nematodes interact with their environment and in particular, how bacterial diet can influence predatory behavior and developmental decisions, such as the mouth form dimorphism (75). I then screened *P. pacificus* survival on all isolated bacterial strains, and identified multiple strains of diverse taxonomic groups that are candidates for nematode pathogens. Among these, the genus *Serratia* has been previously described as a potent killer of *P. pacificus* and *C. elegans* (105), indeed, survival assays showed that one of the *Stenotrophomonas sp.* isolates is also a potential pathogen to *P. pacificus*. It is important to note that in nature *P. pacificus* is exposed to a mixture of bacteria, and therefore survival assays performed with monoxenic cultures of test bacteria are partially artificial. Future studies should aim to study combinations of bacteria simultaneously, thereby mimicking more closely their natural environment. I then performed chemotaxis experiments to examine whether nematodes can recognize pathogenic bacteria. Bacterial classes like *Bacilli* and *Actinobacteria* that decreased nematode survival are avoided by *P. pacificus*, which preferred feeding on *E. coli* OP50. However, *P. pacificus* nematodes were unable to recognize certain pathogenic bacteria such as *Serratia*, indicating that *P. pacificus* can broadly recognize and avoid pathogenic bacteria. Overall, the chemotaxis experiments showed that most natural bacterial isolates are preferred over *E. coli* OP50. This is an interesting result given that the strain PS312 has been fed on *E. coli* in the laboratory since 1988 (52), but apparently has not developed a preference for it. Nevertheless this finding is consistent with observations from *C. elegans* showing that other bacteria such as *Comamonas* are much better food sources than *E. coli* OP50 (52,106). The survival and chemotaxis analysis also showed substantial phylogenetic signal, indicating that related bacteria give rise to a similar response in terms of nematode survival and chemoattraction. Interestingly, both potential groups of pathogens (*Bacilli* and *Actinobacteria*) that can be recognized and avoided by *P. pacificus* are Gram-positive and spore forming bacteria, suggesting that one or multiple features associated or correlated with Gram-positive bacteria and/or spore formation are responsible for the response in nematodes.

Collectively, the bacterial strains that have been described in this study constitute a resource for future studies of interactions between nematodes and bacteria. My findings raise a number of interesting questions for future investigations, e.g. given the substantial variability in survival, how are these patterns reflected in terms of development and other life history traits? Alternatively, which of the isolated bacteria is the best food source for *P. pacificus*?.

Commensal-bacteria influence on *P. pacificus* life-history traits such as, predation and development have never been studied before. In order to avoid unwanted bacterial contamination and examine the effect of single bacterial isolates throughout developmental stages, I fed nematodes with natural bacteria isolates from the germ-free egg stage. I then investigated the effect on mouth form ratio, pharyngeal pumping, and killing behavior by comparing them to standard laboratory cultures grown on *E. coli* OP50. These experiments showed an irrefutable influence on killing behavior. These findings demonstrate a connection between diet and the nervous system in nematodes. Diverse bacterial species had different effects on the predatory behavioral state with some adversely influencing predation while others enhanced the predatory behaviors. The most notable enhancement in predatory behaviors was observed when *P. pacificus* was fed upon *Novosphingobium*. One of the most astonishing predatory behaviors I observed on *P. pacificus* is surplus-killing behavior, where predator *P. pacificus* young adults were killing and abandoning the *C. elegans* larvae corpses without necessarily feeding upon them. Surplus killing behavior has been reported for many predators, however this is the first case observed in nematode species. One of the reasons for the surplus-killing behavior may be the competition between *P. pacificus* and *C. elegans* for food in the nature. On the other hand, St animals are unable to predate, therefore Eu animals may perform mass killing to provide food for St animals of the same cohort (e.g. kin selection). As a microbiologist, it was fascinating to observe the impact of microbes on these complex behaviors. I then implemented whole worm transcriptome analysis on nematodes fed with either *Novosphingobium* or *E.coli* OP50 to examine gene regulation in response to bacterial diet. Whole worm transcriptome analysis showed that many fatty acid metabolism genes responded to diet change. I then created transgenic lines to observe the response to bacterial diet in real-time. Through screening the transgenic lines growing on *Novosphingobium* mutants, I found that metabolic pathways such as purine, pyrimidine and vitamin B12 biosynthesis and nitrogen metabolism modulate nematode host behavior and development. It has been proposed that some *P. pacificus* purine biosynthesis pathway genes are absent, suggesting that *P. pacificus* depends on bacteria for purines (107). Moreover, purines and pyrimidines are fundamental to all life, performing many basic functions for cells: ATP serves as the universal currency of cellular energy, cAMP (Cyclic adenosine

monophosphate) and cGMP (cyclic guanosine monophosphate) are key second messenger molecules, purine and pyrimidine nucleotides are precursors for activated forms of both carbohydrates and lipids, nucleotide derivatives of vitamins are essential cofactors in metabolic processes, and nucleoside triphosphates are the immediate precursors for DNA and RNA synthesis. Thus, disruption of these pathways effects the production of crucial metabolites for bacterial growth, symbiotic bacteria or pathogenic bacteria colonization in host (108,109,110). In addition, GlnD (Bifunctional uridylyltransferase/uridylyl-removing enzyme) plays an important role in the regulation of nitrogen assimilation and metabolism in bacteria (111,112), and controls the levels of glutamine, which may provide an amino group to cobalamin (vitamin B12) in bacteria (113).

Only certain bacterial species including *Novosphingobium* are able to produce vitamin B12, and mutations in the vitamin B12 biosynthesis pathway in *Novosphingobium* modulated predatory feeding and development in the nematode host *P. pacificus*. Previous studies have shown vitamin B12 to be essential for *C. elegans* development, including infertility, growth retardation, and a reduction in lifespan observed in animals deficient in vitamin B12 (44,1114,1115). In contrast, behavioral effects have not been reported, and mechanisms of vitamin B12 deficiency in humans that result in neuropathies are currently unknown. It is important to note that there are two known vitamin B12 dependent pathways in animals, and the modulation of predation and surplus killing in *P. pacificus* requires both vitamin B12-dependent pathways. Therefore, I speculate that the influence of vitamin B12 on these behaviors is multifactorial, and might well involve several factors. Specifically, the SAM (*S*-Adenosyl methionine) pathway feeds into the methylation of DNA, RNA and proteins, but also lipids and neurotransmitters. Thus, the presence of vitamin B12 might act through multiple downstream factors, but how it stimulates these effects has yet to be discovered. Importantly however, several neural circuits and neurotransmitter systems of *P. pacificus* have been investigated (56,82,83,116).

In summary, future studies can reveal the influence of vitamin B12 on fatty acid biosynthesis regulation and modulation of the neurotransmitters such as serotonin and dopamine, which play an important role on nematode behavior. Furthermore, metabolome analysis of the *Novosphingobium* purine, pyrimidine, and vitamin B12 biosynthesis and nitrogen metabolism mutants may provide further information on how mutations in these vital pathways influence bacterial metabolite production, there consequences for host-microbe interactions.

5. Acknowledgments

6. References

1- Lora V. Hooper, and Jeffrey I. Gordon Science 2001;292:1115-1118

2-Simon, C., and R. Daniel 2011. Metagenomic analyses: past and future

Trends. Appl. Environ. Microbial. 77: 1153–1161.

3- Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell. 2005;122:107–118. doi: 10.1016/j.cell.2005.05.007.

4-Boris, S., Suárez, J. E., Vázquez, F., & Barbés, C. (1998). Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. *Infection and immunity*, *66*(5), 1985–1989.

5- Clarke DJ (2008) *Photorhabdus*: a model for the analysis of pathogenicity and mutualism. Cell Microbiol 10: 2159–2167.

6-Abt, M. C., & Pamer, E. G. (2014). Commensal bacteria mediated defenses against pathogens. *Current opinion in immunology*, *29*, 16–22. doi:10.1016/j.coi.2014.03.003

7-Fanning, S., Hall, L. J., Cronin, M., Zomer, A., MacSharry, J., Goulding, D., … van Sinderen, D. (2012). Bifidobacterial surface-exopolysaccharide facilitates commensal-host interaction through immune modulation and pathogen protection. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(6), 2108–2113. doi:10.1073/pnas.1115621109

8- Price, L. B., Hungate, B. A., Koch, B. J., Davis, G. S., & Liu, C. M. (2017). Colonizing opportunistic pathogens (COPs): The beasts in all of us. *PLoS pathogens*, *13*(8), e1006369. doi:10.1371/journal.ppat.1006369

9- omkovich, S., & Jobin, C. (2016). Microbiota and host immune responses: a love-hate relationship. *Immunology*, *147*(1), 1–10. doi:10.1111/imm.12538

10- Lihong Chen, Zhaohui Xiong, Lilian Sun, Jian Yang, Qi Jin, VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors, *Nucleic Acids Research*, Volume 40, Issue D1, 1 January 2012, Pages D641–D645,

11-Masri, L., Branca, A., Sheppard, A. E., Papkou, A., Laehnemann, D., Guenther, P. S., … Schulenburg, H. (2015). Host-Pathogen Coevolution: The Selective Advantage of Bacillus thuringiensis Virulence and Its Cry Toxin Genes. *PLoS biology*, *13*(6), e1002169. doi:10.1371/journal.pbio.1002169

12- V. Briken, Molecular Mechanisms of Host-Pathogen Interactions and their Potential for the Discovery of New Drug Targets. *Current Drug Targets*. **9**, 150–157 (2008).

13-8. Boulange CL, Neves AL, Chilloux J, Nicholson JK, Dumas ME. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Med. 2016;8:42.

14- Zhang, J., Holdorf, A. D., & Walhout, A. J. (2017). C. elegans and its bacterial diet as a model for systems-level understanding of host-microbiota interactions.

Current opinion in biotechnology, *46*, 74–80. doi:10.1016/j.copbio.2017.01.008

15- J. H. F. Van Abeelen, The genetics of behaviour. *NORTH HOLLAND PUBL. CO., AMSTERDAM* (1974).

16- Genome sequence of the nematode C. elegans: A platform for investigating biology. *Science.* **282** (1998), pp. 2012–2018.

17- T.W. Harris, N. Chen, F. Cunningham, M. Tello-Ruiz, I. Antoshechkin, C. Bastiani, T. Bieri, D. Blasiar, K. Bradnam, J. Chan, *et al.*WormBase: a multi-species resource for nematode biology and genomics

Nucleic Acids Res, 32 (2004), pp. D411-D417

18- Grove, C. A., De Masi, F., Barrasa, M. I., Newburger, D. E., Alkema, M. J., Bulyk, M. L., & Walhout, A. J. (2009). A multiparameter network reveals extensive divergence between C. elegans bHLH transcription factors. *Cell*, *138*(2), 314–327. doi:10.1016/j.cell.2009.04.058

19- Khan F, Jain S, Oloketuyi SF (2018) Bacteria and bacterial products: foe and friends to *Caenorhabditis elegans*. Microbiol Res 215:102–113

20-Samuel B.S., Rowedder H., Braendle C., Felix M.A., Ruvkun G. *Caenorhabditis elegans* responses to bacteria from its natural habitats. Proc. Natl. Acad. Sci. USA. 2016;113:E3941–E3949. doi: 10.1073/pnas.1607183113.

21- H. Schulenburg, M. A. Félix, The natural biotic environment of Caenorhabditis elegans. *Genetics*. **206**, 55–86 (2017).

22-C. N. V. Kirienko, B. O. Cezairliyan, F. M. Ausubel, J. R. Powell, Pseudomonas aeruginosa PA14 pathogenesis in *Caenorhabditis elegans*. *Methods Mol. Biol.* **1149**, 653–669 (2014).

23- C. Darby, Interactions with microbial pathogens. *WormBook* (2005), doi:10.1895/wormbook.1.21.1.

24- L. Pardo-López, M. Soberón, A. Bravo, Bacillus thuringiensis insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. *FEMS Microbiol. Rev.* **37** (2013), pp. 3–22.

25- E. Pradel *et al.*, Detection and avoidance of a natural product from the pathogenic bacterium {\lsma} by {\lcel}. *Proc. Natl. Acad. Sci. U. S. A*. **104**, 2295–2300 (2007).

26- A. Osanai, D.-L. Hu, A. Nakane, Caenorhabditis elegans avoids staphylococcal superantigenic toxins via 5-hydroxytryptamine-dependent pathway . *Can. J. Microbiol.* **58**, 1268–1277 (2012).

27- D. Papp, P. Csermely, and C. Söti, "A role for SKN-1/Nrf in pathogen resistance and immunosenescence in *Caenorhabditis elegans*," PLOS Pathogens, vol. 8, no. 4, Article ID e1002673, 2012.

28- T. Roeder *et al.*, Caenopores are antimicrobial peptides in the nematode Caenorhabditis elegans instrumental in nutrition and immunity. *Dev. Comp. Immunol.* **34**, 203–209 (2010).

29- M. Shapira, Host–microbiota interactions in Caenorhabditis elegans and their significance. *Curr. Opin. Microbiol.* **38** (2017), pp. 142–147.

30- S. Brenner,The genetics of Caenorhabditis elegans. *Genetics*. **77**, 71–94 (1974).

31- K. K. Brooks, B. Liang, J. L. Watts, The influence of bacterial diet on fat storage in *C. elegans*. *PLoS One*. **4** (2009), doi: 10.1371/journal.pone.0007545.

32- L. T. MacNeil, E. Watson, H. E. Arda, L. J. Zhu, A. J. M. Walhout, Diet-induced developmental acceleration independent of TOR and insulin in C. elegans. *Cell*. **153**, 240–252 (2013).

33- S. N. Reinke, X. Hu, B. D. Sykes, B. D. Lemire, Caenorhabditis elegans diet significantly affects metabolic profile, mitochondrial DNA levels, lifespan and brood size. *Mol. Genet. Metab.* **100**, 274–282 (2010).

34- B. Virk *et al.*, Excessive folate synthesis limits lifespan in the C. elegans: E. coli aging model. *BMC Biol.* **10** (2012), doi:10.1186/1741-7007-10-67.

35- F. Gomez *et al.*, Delayed accumulation of intestinal coliform bacteria enhances life span and stress resistance in Caenorhabditis elegans fed respiratory deficient E. coli. *BMC Microbiol.* **12** (2012), doi:10.1186/1471-2180-12-300.

36- Y. Kiyama, K. Miyahara, Y. Ohshima, Active uptake of artificial particles in the nematode Caenorhabditis elegans. *J. Exp. Biol.* **215**, 1178–1183 (2012).

37- C. H. Rankin, Nematode behavior: The taste of success, the smell of danger! *Curr. Biol.* **16** (2006), , doi:10.1016/j.cub.2006.01.025.

38- I. Iatsenko, J. J. Yim, F. C. Schroeder, R. J. Sommer, B. subtilis GS67 protects C. elegans from gram-positive pathogens via fengycin-mediated microbial antagonism. *Curr. Biol.* **24**, 2720–2727 (2014).

39- S. Montalvo-Katz, H. Huang, M. D. Appel, M. Berg, M. Shapira, Association with soil bacteria enhances p38-dependent infection resistance in caenorhabditis elegans. *Infect. Immun.* **81**, 514–520 (2013).

40- A. Kamaladevi, K. Balamurugan, Lactobacillus casei triggers a TLR mediated RACK-1 dependent p38 MAPK pathway in Caenorhabditis elegans to resist Klebsiella pneumoniae infection. *Food Funct.* **7**, 3211–3223 (2016).

41- I. Gusarov *et al.*, Bacterial nitric oxide extends the lifespan of C. elegans. *Cell*. **152**, 818–830 (2013).

42- F. H. Nazki, A. S. Sameer, B. A. Ganaie, Folate: Metabolism, genes, polymorphisms and the associated diseases. *Gene*. **533** (2014), pp. 11–20.

43- H. Na, O. Ponomarova, G. E. Giese, A. J. M. Walhout, C. elegans MRP-5 Exports Vitamin B12 from Mother to Offspring to Support Embryonic Development. *Cell Reports*. **22**, 3126–3133 (2018).

44- E. Watson *et al.*, Interspecies systems biology uncovers metabolites affecting C. elegans gene expression and life history traits. *Cell*. **156**, 759–770 (2014).

45- J. Roth, J. Lawrence, T. Bobik, COBALAMIN (COENZYME B 12): Synthesis and Biological Significance . *Annual Review of Microbiology*. **50**, 137–181 (2002).

46- C. Elders, Treatment of pernicious anemia by a special diet. *Journal of the American Medical Association*. **87** (1926), p. 1666.

47- J. R. Roth, J. G. Lawrence, M. Rubenfield, S. Kieffer-Higgins, G. M. Church, Characterization of the cobalamin (vitamin B12) biosynthetic genes of Salmonella typhimurium. *Journal of Bacteriology*. **175**, 3303–3316 (1993).

48-S. J. Moore, M. J. Warren, in *Biochemical Society Transactions* (2012), vol. 40, pp. 581–586.

49-H. Fang, J. Kang, D. Zhang, Microbial production of vitamin B12: A review and future perspectives. *Microbial Cell Factories*. **16** (2017), doi:10.1186/s12934-017-0631-y.

50- Z. Schneider, A. Stroński, *Comprehensive b12: chemistry, biochemistry, nutrition, ecology, medicine* (De Gruyter Mouton, 2011).

51- K. Leishear *et al.*, Relationship between vitamin B12 and sensory and motor peripheral nerve function in older adults. *Journal of the American Geriatrics Society*. **60**, 1057–1063 (2012).

52- S. RJ, C. LK, K. SY, S. PW, Morphological, genetic and molecular description of Pristionchus pacificus sp. n. (Nematoda: Neodiplogastridae). *Fundamental and Applied Nematology*. **19**, 511–522 (1996).

53- J. Srinivasan *et al.*, An integrated physical and genetic map of the nematode Pristionchus pacificus. *Molecular Genetics and Genomics*. **269**, 715–722 (2003).

54- N. Prabh *et al.*, Deep taxon sampling reveals the evolutionary dynamics of novel gene families in Pristionchus nematodes. *Genome Research*. **28**, 1664–1674 (2018).

55- M. S. Werner *et al.*, Young genes have distinct gene structure, epigenetic profiles, and transcriptional regulation. *Genome Research*. **28**, 1675–1687 (2018).

56- R. L. Hong *et al.*, Evolution of neuronal anatomy and circuitry in two highly divergent nematode species (2019).

57- R. L. Hong, R. J. Sommer, Pristionchus pacificus: A well-rounded nematode. *BioEssays*. **28** (2006), pp. 651–659.

58- R. J. Sommer, The future of evo-devo: Model systems and evolutionary theory. *Nature Reviews Genetics*. **10** (2009), pp. 416–422.

59- C. Dieterich, Pristionchus pacificus genomics: from genetics to genome sequence. *WormBook* (2006), doi:10.1895/wormbook.1.116.1.

60- B. Schlager, X. Wang, G. Braach, R. J. Sommer, Molecular cloning of a dominant roller mutant and establishment of DNA-mediated transformation in the nematode Pristionchus pacificus. *Genesis*. **47**, 300–304 (2009).

61- H. Tian, B. Schlager, H. Xiao, R. J. Sommer, Wnt Signaling Induces Vulva Development in the Nematode Pristionchus pacificus. *Current Biology*. **18**, 142–146 (2008).

62- H. Witte *et al.*, Gene inactivation using the CRISPR/Cas9 systemin the nematode Pristionchus pacificus. *Development Genes and Evolution*. **225**, 55–62 (2014).

63- A. Sinha, R. J. Sommer, C. Dieterich, Divergent gene expression in the conserved dauer stage of the nematodes Pristionchus pacificus and Caenorhabditis elegans. *BMC Genomics*. **13** (2012), doi:10.1186/1471-2164-13-254.

64- N. Prabh, C. Rödelsperger, Are orphan genes protein-coding, prediction artifacts, or non-coding RNAs? *BMC Bioinformatics*. **17** (2016), doi:10.1186/s12859-016-1102-x.

65- R. J. Sommer, A. McGaughran, The nematode Pristionchus pacificus as a model system for integrative studies in evolutionary biology. *Molecular Ecology*. **22** (2013), pp. 2380–2393.

66-A. Ogawa, R. J. Sommer, Strategies to get arrested. *Science*. **326** (2009), pp. 944–945.

67- M. Herrmann, W. E. Mayer, R. J. Sommer, Nematodes of the genus Pristionchus are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology*. **109**, 96–108 (2006).

68- M. G. Mayer, R. J. Sommer, Natural variation in Pristionchus pacificus dauer formation reveals cross-preference rather than self-preference of nematode dauer pheromones. *Proceedings of the Royal Society B: Biological Sciences*. **278**, 2784–2790 (2011).

69- R. J. Sommer, in *Nematology Monographs and Perspectives* (Entomological Society of Canada, 2015), vol. 11, pp. 19–41.

70- M. Herrmann, S. Kienle, J. Rochat, W. E. Mayer, R. J. Sommer, Haplotype diversity of the nematode Pristionchus pacificus on Réunion in the Indian Ocean suggests multiple independent invasions. *Biological Journal of the Linnean Society*. **100**, 170–179 (2010).

71- K. Morgan *et al.*, Multi locus analysis of Pristionchus pacificus on la Réunion Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare out-crossing. *Molecular Ecology*. **21**, 250–266 (2012).

72- G. Bento, A. Ogawa, R. J. Sommer, Co-option of the hormone-signalling module dafachronic acid-DAF-12 in nematode evolution. *Nature*. **466**, 494–497 (2010).

73- A. F. Von Lieven, Functional morphology and evolutionary origin of the three-part pharynx in nematodes. *Zoology*. **106**, 183–201 (2003).

74- V. Susoy, E. J. Ragsdale, N. Kanzaki, R. J. Sommer, Rapid diversification associated with a macroevolutionary pulse of developmental plasticity. *eLife*. **2015**, 1–39 (2015).

75- E. J. Ragsdale, M. R. Müller, C. Rödelsperger, R. J. Sommer, XA developmental switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*. **155**, 922 (2013).

76- N. Bose *et al.*, Complex small-molecule architectures regulate phenotypic plasticity in a nematode. *Angewandte Chemie - International Edition*. **51**, 12438–12443 (2012).

77- M. R. Kieninger *et al.*, The Nuclear Hormone Receptor NHR-40 Acts Downstream of the Sulfatase EUD-1 as Part of a Developmental Plasticity Switch in Pristionchus. *Current Biology*. **26**, 2174–2179 (2016).

78-B. Sieriebriennikov *et al.*, A Developmental Switch Generating Phenotypic Plasticity Is Part of a Conserved Multi-gene Locus. *Cell Reports*. **23**, 2835-2843.e4 (2018).

79- V. Serobyan *et al.*, Chromatin remodelling and antisense-mediated up-regulation of the developmental switch gene eud-1 control predatory feeding plasticity. *Nature Communications*. **7** (2016), doi:10.1038/ncomms12337.

80- H. Sato *et al.*, The stomal morphology of parasitic females of Strongyloides spp. by scanning electron microscopy. *Parasitology Research*. **102**, 541–546 (2008).

81- S. Straud, I. Lee, B. Song, L. Avery, Y. J. You, The jaw of the worm: GTPase-activating protein EAT-17 regulates grinder formation in Caenorhabditis elegans. *Genetics*. **195**, 115–125 (2013).

82-M. Okumura, M. Wilecki, R. J. Sommer, Serotonin drives predatory feeding behavior via synchronous feeding rhythms in the nematode Pristionchus pacificus. *G3: Genes, Genomes, Genetics*. **7**, 3745–3755 (2017).

83-M. Wilecki, J. W. Lightfoot, V. Susoy, R. J. Sommer, Predatory feeding behaviour in Pristionchus nematodes is dependent on phenotypic plasticity and induced by serotonin. *Journal of Experimental Biology*. **218**, 1306–1313 (2015).

84-J. Y. Szø, M. Victor, C. Loer, Y. Shi, G. Ruvkun, Food and metabolic signalling defects in a Caenorhabditis elegans serotonin-synthesis mutant. *Nature*. **403**, 560–564 (2000).

85-B. mi Song, L. Avery, Serotonin activates overall feeding by activating two separate neural pathways in Caenorhabditis elegans. *Journal of Neuroscience*. **32**, 1920–1931 (2012).

86-H. Kruuk, Surplus killing by carnivores. *Journal of Zoology*. **166**, 233–244 (1972).

87- B. Zimmermann, H. Sand, P. Wabakken P, O. Liberg, H. P. Andreassen H. P, Predator-dependent functional response in wolves: from food limitation to surplus killing. *J Anim Ecol*. **84**, 102-12 (2015).

88-T. E. Reimchen, Some ecological and evolutionary aspects of bear-salmon interactions in coastal British Columbia. *Can. J. Zool*. **78**, 448–457 (2000).

89- K. Gaydos, S. Raverty, R. W. Baird, R. W. Osborne, Suspected surplus killing of harbor seal pups (*Phoca vitulina*) by killer whales (*Orcinus orca*). *Northwest. Nat*. **86**, 150–154 (2005).

90- G. L. Nunn, D. Klem, T. Kimmel, T. Merriman T, Surplus killing and caching by American kestrels (*Falco sparverius*). *Anim. Behav*. **24**, 759–763 (1976).

91- L. Vesely, D. S. Boukal, M. Buric, P. Kozak, A. Kouba, A. Sentis, Effects of prey density, temperature and predator diversity on nonconsumptive predator-driven mortality in a freshwater food web. *Scientific Reports* **7**, 18075 (2017).

92- J. L. Maupin, S. E. Riechert SE, Superfluous killing in spiders: a consequence of adaptation to food-limited environments? *Behavioral Ecology*, **12**, 569–576 (2001).

93- L. P. Lounibos, S. Makhni, B. W. Alto, Kesavaraju 2008. Surplus killing by predatory larvae of Corethrella appendiculate: prepupal timing and site-specific attack on mosquito prey. *J Ins. Beh.*, **21**, 47-54 (2008).

94- P. Trubl, V. Blackmore, J. C. Johnson, Wasteful killing in urban black widows: gluttony in response to food abundance, *Ethology*, **117**, 236-245 (2011).

95- A. E. Lincoln, T. P. Quinn, Optimal foraging or surplus killing: Selective consumption and discarding of salmon by brown bears. *Behavioral Ecology*. **30**, 202–212 (2019).

96- J. W. Lightfoot *et al.*, Small peptide–mediated self-recognition prevents cannibalism in predatory nematodes. *Science*. **364**, 86–89 (2019).

97-R. Rae *et al.*, Isolation of naturally associated bacteria of necromenic Pristionchus nematodes and fitness consequences. *Journal of Experimental Biology*. **211**, 1927–1936 (2008).

98-C. Dieterich *et al.*, The *Pristionchus pacificus* genome provides a unique perspective on nematode lifestyle and parasitism. *Nature Genetics*. **40**, 1193–1198 (2008).

99- J. Z. Wei *et al.*, Bacillus thuringiensis crystal proteins that target nematodes. *Proceedings of the National Academy of Sciences of the United States of America*. **100**, 2760–2765 (2003).

100- R. Rae, H. Witte, C. Rödelsperger, R. J. Sommer, The importance of being regular: Caenorhabditis elegans and Pristionchus pacificus defecation mutants are hypersusceptible to bacterial pathogens. *International Journal for Parasitology*. **42**, 747–753 (2012).

101- S. Alper *et al.*, The Caenorhabditis elegans germ line regulates distinct signaling pathways to control lifespan and innate immunity. *Journal of Biological Chemistry*. **285**, 1822–1828 (2010).

102- R. Rae, A. Sinha, R. J. Sommer, Genome-Wide Analysis of Germline Signaling Genes Regulating Longevity and Innate Immunity in the Nematode Pristionchus pacificus. *PLoS Pathogens*. **8** (2012)

103- N. Akduman, C. Rödelsperger, R. J. Sommer, Culture-based analysis of Pristionchus-associated microbiota from beetles and figs for studying nematode-bacterial interactions. *PLoS ONE*. **13** (2018), doi:10.1371/journal.pone.0198018.

104- **N. Akduman,** C. Rödelsperger, J. W. Lightfoot, W. Röseler, H. Witte, W-S. Lo, R. J. Sommer, BioRxiv (2019) doi: https://doi.org/10.1101/797803 (104)

105- A. Sinha, R. Rae, I. Iatsenko, R. J. Sommer, System Wide Analysis of the Evolution of Innate Immunity in the Nematode Model Species Caenorhabditis elegans and Pristionchus pacificus. *PLoS ONE*. **7** (2012), doi:10.1371/journal.pone.0044255.

106- B. B. Shtonda, L. Avery, Dietary choice behavior in Caenorhabditis elegans. *Journal of Experimental Biology*. **209**, 89–102 (2006).

107-R. Tyagi, B. A. Rosa, W. G. Lewis, M. Mitreva, Pan-phylum Comparison of Nematode Metabolic Potential. *PLoS Neglected Tropical Diseases*. **9** (2015), doi:10.1371/journal.pntd.0003788.

108- C. L. Shaffer *et al.*, Purine biosynthesis metabolically constrains intracellular survival of uropathogenic Escherichia coli. *Infection and Immunity*. **85** (2017), doi:10.1128/IAI.00471-16.

109- J. K. Kim *et al.*, Purine biosynthesis-deficient Burkholderia mutants are incapable of symbiotic accommodation in the stinkbug. *ISME Journal*. **8**, 552–563 (2014).

110- H. J. Yang, L. Bogomolnaya, M. McClelland, H. Andrews-Polymenis, De novo pyrimidine synthesis is necessary for intestinal colonization of *Salmonella Typhimurium* in chicks. *PLoS ONE*. **12** (2017), doi:10.1371/journal.pone.0183751.

111- Y. Zhang, E. L. Pohlmann, J. Serate, M. C. Conrad, G. P. Roberts, Mutagenesis and functional characterization of the four domains of GlnD, a bifunctional nitrogen sensor protein. *Journal of Bacteriology*. **192**, 2711–2721 (2010).

112- M. R. Atkinson, A. J. Ninfa, Characterization of the GlnK protein of Escherichia coli. *Molecular Microbiology*. **32**, 301–313 (1999).

113- A. A. Yeliseev *et al.*, Glutamine as the source of amide groups in vitamin B-12 biosynthesis. *BBA - General Subjects*. **880**, 131–138 (1986).

114- T. Bito, Y. Matsunaga, Y. Yabuta, T. Kawano, F. Watanabe, Vitamin B 12 deficiency in Caenorhabditis elegans results in loss of fertility, extended life cycle, and reduced lifespan. *FEBS Open Bio*. **3**, 112–117 (2013).

115-H. Na, O. Ponomarova, G. E. Giese, A. J. M. Walhout, C. elegans MRP-5 Exports Vitamin B12 from Mother to Offspring to Support Embryonic Development. *Cell Reports*. **22**, 3126–3133 (2018).

116- D. J. Bumbarger, M. Riebesell, C. Rödelsperger, R. J. Sommer, System-wide rewiring underlies behavioral differences in predatory and bacterial-feeding nematodes. *Cell*. **152**, 109–119 (2013).

7. Appendix

7.1 Supplementary Table 1: List of natural bacteria isolates

Location	Phylum	Class	Order	Family	Genus
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	2-Serratia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	3-Acinetobacter
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	4-Proteus vulgaris
LR beetle	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	6-Achromobacter
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	7-Serratia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	8-Pseudomonas sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	10-Bacillus cereus
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	11-Chryseobacterium s
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	12-Bacillus sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	13-Bacillus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	14-Acinetobacter sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	16-Staphylococcus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	17-Hafnia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	18-Stenotrophomonas s
LR beetle	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	19-Stenotrophomonas s
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	20-Morganella sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	21-Acinetobacter sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	22-Bacillus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	26-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	28-Comamonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	29-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	31-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	32-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	33-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	34-Enterobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	36-Morganella sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	39-Bacillus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	40-Serratia sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	41-Exiguobacterium sp
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	44-Providencia sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	45_Chryseobacterium s
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	46-Cronobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	47-Pseudomonas sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	49-Bacillus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	50-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	53-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	56-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	59-Acinetobacter sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Planococcaceae	62-Kurthia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	63-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	65-Pseudomonas sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	66-Bacillus cereus

Location	Phylum	Class	Order	Family	Genus
LR beetle	Firmicutes	Bacilli	Bacillales	Planococcaceae	68-Kurthia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	69-Serratia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	70-Serratia sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	71-Staphylococcus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	72-Serratia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	73-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	74-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	75-Yokenella sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	76-Enterobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	80-Acinetobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	81-Morganella sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	83-Pseudomonas sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	86-Bacillus sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	88-Pseudomonas prote
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	89-Wautersiella sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	90-Acinetobacter sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	93-Chryseobacterium s
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	94-Pseudomonas sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	96-Hafnia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	98-Enterobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	102-Acinetobacter sp.
LR beetle	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	103-Comamonas sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	104-Wautersiella sp.
LR beetle	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	106-Leucobacter sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	108-Wautersiella sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	109-Wautersiella sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	111-Proteus vulgaris
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	112-Proteus sp.
LR beetle	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	113-Acinetobacter sp.
LR beetle	Firmicutes	Bacilli	Bacillales	Bacillaceae	115-Bacillus cereus
LR beetle	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	116-Micrococcineae sp
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	118-Serratia sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	119-Serratia sp.
LR beetle	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	120-Chryseobacterium
LR beetle	Actinobacteria	Actinobacteria	Actinobacteridae	Actinomycetales	122-Micrococcineae sp
LR beetle	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	97a-Enterobacter sp.
LR beetle	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	97b-Acinetobacter sp.
LR fig	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	L2-Sphingobacterium s
LR fig	Actinobacteria	Actinobacteria	Micrococcales	Micrococcaceae	L6-Micrococcineae sp.
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	L18-Delftia sp.
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	L21-Achromobacter sp
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	L23-Xenophilus sp.
LR fig	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	L25-Chryseobacterium
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	L26-Shinella sp.

Location	Phylum	Class	Order	Family	Genus
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	L28-Xenophilus sp.
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	L-29-Variovorax sp.
LR fig	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	L31-Stenotrophomonas
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	L35-Achromobacter sp
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	L41-Achromobacter sp
LR fig	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	L43-Stenotrophomonas
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	L48-Rhizobium sp.
LR fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	L53-Enterobacter sp.
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	L54-Rhizobium sp.
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	L55A-Variovorax sp.
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	L56-Kaistia sp.
LR fig	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	L74-Azotobacter sp.
LR fig	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	L76-Novosphingobium
LR fig	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	L78-Novosphingobium
LR fig	Firmicutes	Bacilli	Bacillales	Bacillaceae	L82-Bacillus sp.
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	L91-Achromobacter sp
LR fig	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	L93-Azorhizobium sp.
LR fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	L100-Achromobacter s
Vietnem fig	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	V6-Sphingomonas sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V8-Escherichia-Shigell
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V-14-Klebsiella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V15-Pectobacterium sp
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V20-Escherichia-Shige
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V27-Raoultella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V39-Enterobacter sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	V46-Acinetobacter sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V48-Raoultella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V52-Pectobacterium sp
Vietnem fig	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	V61-Xenophilus sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V64-Klebsiella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V69-Enterobacter sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	V71-Xanthomonas sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V72-Escherichia-Shige
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V74-Klebsiella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V88-Escherichia-Shige
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V91-Enterobacternone
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V94-Enterobacter sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V97-Klebsiella sp.
Vietnem fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	V100-Pantoea sp.
S. Africa fig	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	LB4A-Pseudomonas sp
S. Africa fig	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	LB6-Rhodobacter sp.
S. Africa fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA6-Erwinia sp.
S. Africa fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NGM-2B-Raoultella sp
S. Africa fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NGM-5-Raoultella sp.
S. Africa fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NGM6A-uncultured sp

Location	Phylum	Class	Order	Family	Genus
S. Africa fig	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	TSA3A-Acinetobacter
S. Africa fig	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	YPD6-Tatumella sp.