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May the worm story begin…

Evaluation of *Caenorhabditis elegans* as model for investigations into bacterial cross talk during intestinal immune responses

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Abbreviations

BHI	Brain Heart Infusion
CD	Crohn's Disease
CDAD	*Clostridium difficile*-associated diarrhea
CGC	Caenorhabditis genetics center
ESP	enhanced susceptibility to pathogens
FACS	fluorescence-activated cell sorting
GALT	gut-associated lymphoid tissue
GI tract	gastrointestinal tract
HRP	Horseradish Peroxidase
IBD	Inflammatory Bowel Disease
LB	Lysogeny Broth
MAP	Mitogen-activated protein
NGM	Nematode Growth Medium
PFA	Paraformaldehyde
RFU	relative Fluorescent Unit
SAPLIP	Saposin-like Protein
SEM	standard error of the mean
SD	standard deviation
SOD-3	Superoxid Dismutase 3
TBE	Tris-Borate-EDTA
TLR	Toll like Receptor
UC	Ulcerative Colitis

1 Introduction

1.1 Background

Microorganisms are critical for health and disease and play a key role in the existence of their host in many species. For humans the positive contributions of gut microorganisms are well known and have led to the term of "a forgotten organ" (O'Hara and Shanahan 2006) and the initiation of the 'Human Microbiome Project'. Examples of positive influences of host fitness include reproduction (Shimizu, Muranaka et al. 1998), development (Piper, Blanc et al. 2014), metabolism (Turnbaugh, Ley et al. 2006), lifespan (Biagi, Franceschi et al. 2016) and immunity (Round and Mazmanian 2009). Although an important role of symbiotic microorganisms in the regulation of host fitness can be shown for many species (Zilber-Rosenberg and Rosenberg 2008) the molecular mechanisms underlying host-microbe crosstalk are largely unknown.

The biggest challenge is the immense numbers of symbiotic microbes underlying these interactions (Zilber-Rosenberg and Rosenberg 2008), which makes it difficult to find an appropriate model. Simple model organisms are therefore a good tool for investigating many aspects of how bacteria affect physiology in health and disease. *Caenorhabditis elegans* is one of the most thoroughly studied animal models in many areas of research. Due to its excellent genetics, large research community, and its relative simplicity compared to rodents, it is an excellent model to study the effects of microorganisms on physiology. Equally important is the fact that it can easily be maintained monoxenically (i.e. with one bacterial species), which simplifies research into microbe-host interactions. One interesting and increasingly popular aspect regarding microorganisms is their contribution to host physiology and immunity. Symbiotic microorganisms, especially intestinal microorganisms, often play key roles in the development and efficacy of their host's immune system. The importance of these contributions can manifest clinically in a variety of diseases and health problems including obesity, colon cancer, allergies or inflammatory bowel disease (IBD).

1.2 Etiology and Pathogenesis of Inflammatory bowel diseases

The two major manifestations of IBD are Crohn's Disease (CD) and ulcerative colitis (UC) both of which are characterized by recurring idiopathic inflammation (Loh and Blaut 2012). In the case of UC inflammation is restricted to the superficial layers, mucosa

and submucosa, of the rectum or colon, whereas CD may affect the entire gastrointestinal (GI) tract anywhere from mouth to anus and is not limited to superficial layers but may affect the colonic wall transmurally (Yang, Taylor et al. 2001, Loh and Blaut 2012). Clinical symptoms of UC and CD include fever, diarrhea, rectal bleeding, and signs of malnutrition (Podolsky 2002). Currently treatment of both diseases involves immunosuppressive agents combined with a carefully observed diet, and for severe disease presentation surgery (Lin and Hackam 2011). These therapies are alleviating; however, they are not curative due to the still not fully understood etiology of IBD. Several risk factors including genetic susceptibility, the host's immune system, and the individual microbiota are thought to interplay in order to progress disease manifestation. These factors seem to be implicated in disease pathogenesis by deregulating host–bacterial interactions. To break down the complexity of this interplay the contributing components will be introduced individually in the following sections:

1.2.1 Intestinal inflammation and the genetic susceptibility

Several studies have shown an increase from 10- to 30-fold in IBD prevalence among siblings (Orholm, Munkholm et al. 1991, Binder 1998, Orholm, Fonager et al. 1999), 30-50% concordance for IBD among monozygotic twins (Thompson, Driscoll et al. 1996, Orholm, Binder et al. 2000, Halfvarson, Bodin et al. 2003) as well as higher disease frequencies between monozygotic than dizygotic twins (Tysk, Lindberg et al. 1988, Halme, Paavola-Sakki et al. 2006) indicating a genetic component of disease susceptibility. Nevertheless, not all of monozygotic twins develop IBD pointing to a reduced genetic penetrance and the implication of further disease determinants like environmental factors.

Genome-wide association studies (GWAS) are a useful tool for identifying of shared gene variants in individuals with a common trait. Indeed, recent GWAS were able to identify common risk variants associated with IBD development. These variants include genes encoding for the nucleotide-binding oligomerization domain-containing protein 2 (NOD2), the toll-like receptor 4 (TLR4) and the autophagy-related 16-like 1 protein (ATG16L1) (Kaser, Zeissig et al. 2010, Thompson and Lees 2011). Variants in UC-specific susceptibility genes include interleukin-10 (IL-10), the extracellular matrix protein 1 (ECM1*)* or cadherin-1 (CDH1) (Thompson, Driscoll et al. 1996) and susceptibility for CD include signal transducer and activator of transcription 3 (STAT3),

protein tyrosine phosphatase, nonreceptor types 2 and 22 (PTPN2 and PTPN22) (Barrett, Hansoul et al. 2008). Common to all these genes is their role in immune response modulation pointing to an immunological component in disease formation.

1.2.2 Intestinal inflammation and the host's intestinal immune system

The main task of the intestinal immune system is to provide an intestinal barrier that allows the host's commensal microorganisms to *integrate* while protecting against invasion of pathogenic bacteria in to blood and lymph (Magalhaes, Tattoli et al. 2007). The intestinal system comprises different components to protect against pathogens: The intestinal mucosa forms a physical barrier with its mucus layer as well as a biological barrier with its antimicrobial peptides such as IgA antibodies. Microorganisms also are kept at bay by the gut-associated lymphoid tissue (GALT). Nevertheless, a certain amount of potentially harmful bacteria is tolerated in a healthy intestinal system without pathogenic side effects: different bacteria species compete for limited resources within the GI tract such as space and nutrients. Beneficial bacteria of the gut flora are well adapted to this competitive niche and can serve to prevent overgrowth of potentially pathogenic bacteria. If this intestinal homeostasis is disrupted it can clinically manifest for example *Clostridium difficile*-associated diarrhea (CDAD): *C. difficile* is a potentially pathogenic anaerobic bacterium which is present in the human colon in 2% of the adult, non-hospitalized population (Poutanen and Simor 2004). After antibiotic therapy which disrupts the homeostasis of the gut flora, *C. difficile* may opportunistically dominate, causing CDAD. Besides antibiotic therapy inflammation may contribute or exacerbate a dysregulation of intestinal inflammation: inflammation supports for example growth of S*almonella enterica* serovar Typhimurium (*S.* Typhimurium) (Thiennimitr, Winter et al. 2011): it was shown that ethanolamine, a specific nutrient, gives a growth advantage in the inflamed intestine of *S.* Typhimurium. For most bacteria ethanolamine supports no growth by fermentation in the anerobic environment of the gut. *S.* Typhimurium, however, carries a unique gene set, the *eut* gene cluster, that allows for degradation and utilization of ethanolamine thereby supporting anaerobic growth on ethanolamine.

1.2.3 Intestinal inflammation and the microbiota

For CD and UC patients it has been shown that the disease can be diagnosed based on the microbiota composition in their feces (Swidsinski, Loening-Baucke et al. 2008). Ott and colleagues showed that the biodiversity in microbiota of healthy controls is twofold that of patients suffering from CD and threefold that of patients suffering from UC (Ott, Mustdelft et al. 2004). Further they showed that the total number of bacterial cells did not differ between IBD patients and healthy controls but quantification of anaerobic bacteria of the taxa Firmicutes and Bacteroidetes showed a significant reduction in cell numbers in the patient group. This change within the intestinal microbiome with a reduction in obligate anaerobes of Firmicutes and an increase in facultative anaerobes of Proteobacteria has led to the proposal of a putative "oxygen" hypothesis suggesting that disruption of anaerobiosis and more precisely presence of oxygen contributes to intestinal dysbiosis associated with IBD (Rigottier-Gois 2013). Patients with other inflammatory diseases accompanied by diarrhea show a bacterial diversity that is comparable to that of non-inflammatory controls and which is significantly higher than that of IBD patients. Moreover, an increase of *Escherichia coli* including variants that are pathogenic have been documented in ileal CD (Darfeuille-Michaud, Neut et al. 1998, Willing, Halfvarson et al. 2009).

Further evidence pointing to a role of the intestinal microbiota in the development of IBD is that IBD patients are significantly more likely to have been prescribed antibiotics two to five years prior to their diagnosis and that there is a dose-dependent association between adult-onset of IBD and antibiotic use (Shaw, Blanchard et al. 2011).

Taken together these findings suggest reduced bacterial diversity to be a disease specific feature of IBD (Ott, Mustdelft et al. 2004).

1.3 *C. elegans* as model organism for intestinal host–microbiota interactions

In his Nobel Prize speech Sydney Brenner described *Caenorhabditis elegans* as "Nature's gift to Science" (Brenner 2002). And indeed since its introduction in 1974 by Sydney Brenner it has proven to be a well suited model organism for investigations into many central processes, immunity (Nicholas and Hodgkin 2004), like energy metabolism (Zheng and Greenway 2012) and ageing (Murphy, McCarroll et al. 2003). This success

is mainly owed to its excellent genetics, including a fully sequenced genome, its short lifespan (2–3 weeks) as well as readily available mutants, and its transparency, which allows for easy visualization of its internal organs as well as fluorescently tagged cells (Lin and Hackam 2011). Conveniently gene expression in *C. elegans* can be knocked down by double-stranded RNA (dsRNA)-mediated RNA interference by providing worms live non-pathogenic *E. coli* that are expressing dsRNA corresponding to the gene of interest (Boutros and Ahringer 2008).

1.4 Anatomy and life cycle of *C. elegans*

C. elegans is a small free-living soil nematode with a length around 1 mm. The majority of the population is made up by hermaphrodites (XX) with a small number of males (X0). The hermaphrodite is self-fertilizing by producing both eggs and sperm, resulting in a progeny of up to 300 worms (Ann K. Corsi 2015). The body structure of an adult worm consists of an outer and inner tube and is unsegmented (see figure 1). The outer tube is made up of the cuticle, a hypodermis, an excretory system as well as muscles and neurons, while the inner tube is made up of a pharynx, the intestine and the gonads. Ganglia in head and tail together with a ventral and a lateral nerve cord make up *C. elegans'* nervous system (Ann K. Corsi 2015).

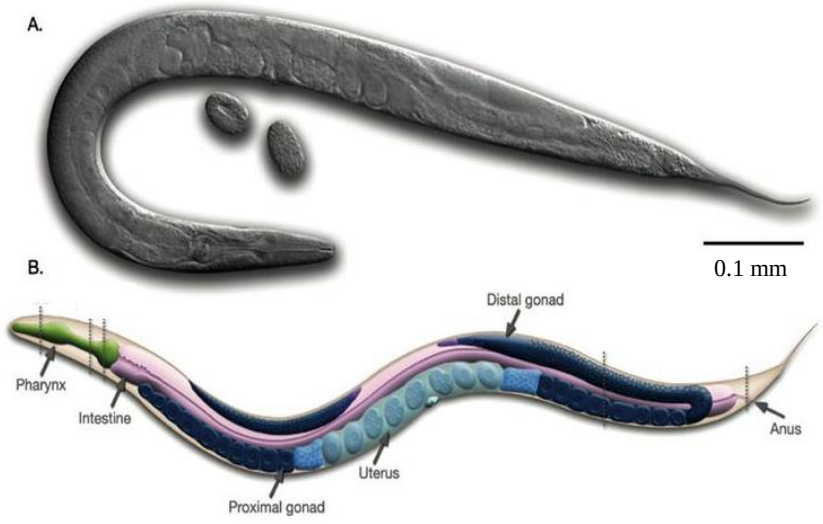

Figure 1. Anatomy of an adult hermaphrodite. A. Differential interference contrast (DIC) image of an adult hermaphrodite **B.** Schematic drawing of anatomical structures (Altun and Hall 2015).

The worm has four larval stages (see Figure 2). It develops into an adult over the course of three days, and has a lifespan of approximately three weeks at 20°C. Under unfavorable conditions the larva will go into Dauer stage. Animals enter this state upon starvation, over-crowding, or upon the production of a Dauer hormone (Golden and Riddle 1982). In the Dauer stage, larvae are hyper-resistant to stress and interestingly they do not age (Kenyon, Chang et al. 1993). In 1998 the genome sequence of *C. elegans* was completed as the first multicellular organism (1998).

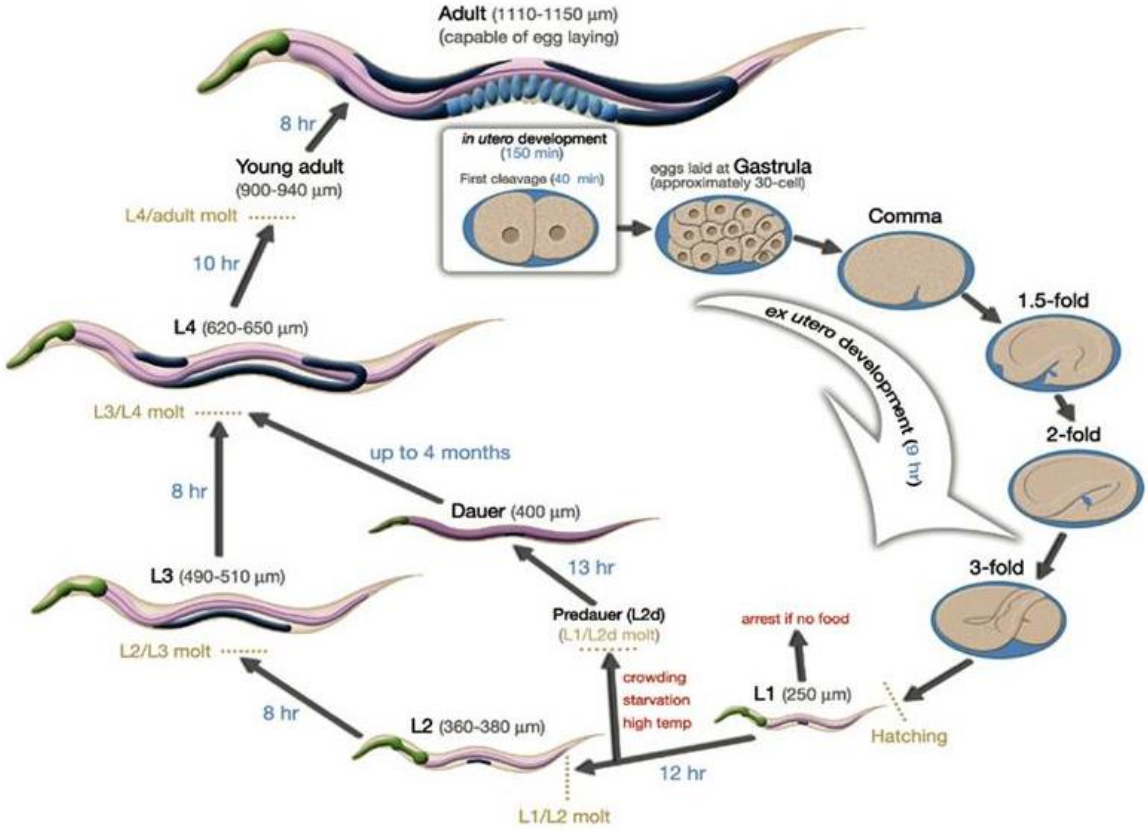

Figure 2. Life cycle of *C. elegans* at 22°C; 0 minute is fertilization. Eggs, during the gastrula stage, are laid outside at about 150 minutes after fertilization. Blue numbers indicate the length of time at a certain stage. The length of the animal is noted in micrometers (µm) next to the stage name (Altun and Hall 2015).

1.4.1 *C. elegans* and its defense system

C. elegans is protected by strong physical barriers, the cuticle and the grinder, which disrupts bacteria (Portal-Celhay and Blaser 2012). Further it was shown that the sole Toll like Receptor (TLR) of *C. elegans*, TOL-1 (Table 1), additionally helps to prevent bacterial invasion of the pharynx (Tenor and Aballay 2008). However, these barriers do

not protect completely against microbial entry, and some can pass through the grinder and colonize the intestine (Tan, Mahajan-Miklos et al. 1999).

Besides this first line of defense there are further mechanisms against pathogens. However, it appears that *C. elegans* does not have specialized immune cells like phagocytes (Irazoqui, Urbach et al. 2010). Instead it uses aversive olfactory learning to avoid pathogens making up a form of adaptive immunity using behavior (Zhang, Lu et al. 2005). Upon bacterial or fungal infection many effector genes can also be upregulated in C. *elegans* (Shivers, Youngman et al. 2008). Effector genes are for example genes that play part in intracellular defense, that can inactivate intracellular toxins, and that can eliminate toxins out of the cell. *C. elegans* also possesses a large variety of genes that are responsible for extracellular defense. In fact around 17% of the genes in the genome of *C. elegans* are thought to encode proteins that are secreted, and 30% of these genes are upregulated when exposed to pathogenic bacteria (Suh and Hutter 2012, Balla and Troemel 2013). Often these factors belong to larger gene families and include various proteins conserved within mammals, like saposin-like proteins (SAPLIP), which have anti-microbial activity, while others are nematode-specific. Screening *C. elegans* mutants with enhanced susceptibility to infection by certain pathogens, several esp genes (esp = enhanced susceptibility to pathogens) were identified like esp-8 (NSY-1), coding for mitogen-activated protein (MAP) kinase kinase kinase, and esp-2 (SEK-1), coding for a MAP kinase kinase (Kim, Feinbaum et al. 2002). Furthermore, the RNA interference knockdown of pmk-1, encoding one of two MAP kinases in *C. elegans* (Table 1) results in a strong esp phenotype (Kim, Feinbaum et al. 2002), indicating that this pathway is normally protective against pathogens. MAP kinases play an important role in the mammalian cellular immune response to lipopolysaccarides (LPS) demonstrating that *C. elegans* possesses conserved and ancient innate immune pathways (Dong et al., 2002). Further evolutionary conserved signaling pathways for innate immunity include the DAF-2 insulin/IGF-like receptor (Insulin-like growth factors) (Evans, Chen et al. 2008, Evans, Kawli et al. 2008). Daf-2, a transmembrane tyrosine kinase insulin receptor, activates a complex neuroendocrine signaling pathways that negatively regulates the transcription factor DAF-16 (Table 1) (Kenyon, Chang et al. 1993). Upon activation by agonist binding to the DAF-2 receptor, DAF-16 is phosphorylated resulting in its retention in the cytoplasm and repression of its downstream signaling (Lin, Hsin et al.

2001). On the other hand upon activation of DAF-16 hundred of genes are expressed including various factors involved in defense against pathogens such as resistance to oxidative stress (superoxidase dismutase, glutathione-S-transferase, catalase), detoxification (e.g. metallothioneins) and general stress responses (heat shock proteins) (Gravato-Nobre and Hodgkin 2005). Microarray technology has been used to identify several antimicrobial targets of DAF-16, e.g. the lysozyme genes *lys-7* and *lys-8* and saposin genes including *spp-1* (Table 1) (Murphy, McCarroll et al. 2003). While some components are conserved others, such as NFκB of the toll pathway seem to be absent in the worm's genome.

Table 1. Selected enzymes of the *C. elegans* defense system. Information on gene function was obtained from www.wormbase.org; further information can be found in the text.

Gene name	Function
Daf-16	daf-16 encodes the sole *C. elegans* forkhead box O (FOXO) transcription factor that acts in the insulin/IGF-1-mediated signaling (IIS) pathway. In *C.elegans* this pathway is involved in Dauer formation, longevity, stress response, and innate immunity.
Spp-1	spp-1 encodes a member of the SAPLIP superfamily; the expression of SPP-1 is regulated by the DAF-2/insulin receptor signaling pathway and by the presence of bacterial pathogens.
Lys-7	lys-7 encodes an antimicrobial lysozyme which functions in the innate immune response; loss of lys-7 activity results in enhanced susceptibility to *Pseudomonas aeruginosa*, but enhances tolerance to the enteric bacteria *Salmonella* Typhimurium
Tol-1	tol-1 encodes a transmembrane protein that is the only *C. elegans* TLR; TOL-1 is required for correct embryonic development and pathogen avoidance behavior; it is mainly expressed in the nervous system.
Pmk-1	pmk-1 encodes a MAP kinase (orthologous to human p38 MAPK) that regulates the innate immune response; PMK-1 physically interacts with the ATF-7 transcription factor to regulate the transcriptional innate immune response.

1.4.2 Commensal microbiota and host–microbiota interactions of *C. elegans*

When isolated from the wild, *C. elegans* often harbors a diverse bacterial flora in its gut lumen, and in a study mimicking a natural environment of soil and rotten fruit 18 gut-associated bacterial species have been identified (Montalvo-Katz, Huang et al. 2013). In the laboratory *C. elegans* is usually grown in the presence of one single bacterial strain (Brenner 1974); usually this is *Escherichia coli*, a gram negative bacterium, but other bacteria species can also be used. Initially the *E. coli* with which *C. elegans* is usually cultured was simply regarded as a food source, but more recently it was discovered to be slightly pathogenic (Gems and Riddle 2000). Much like humans, an adult worm is made up by approximately 10,000 bacteria, which is 10- times higher than that of the worm's own somatic cells (Portal-Celhay, Bradley et al. 2012).

Similarly to humans in *C. elegans* the intestinal bacteria composition has been shown to profoundly affect many aspects of the worm's physiology such as its lifespan (Cabreiro, Au et al. 2013, Komura, Ikeda et al. 2013), metabolome (Cabreiro, Au et al. 2013), transcriptome (Watson, MacNeil et al. 2013), and intestinal fat storage (Brooks, Liang et al. 2009). Furthermore if *C. elegans* is provided with a complete but bacteria-free diet, its development is strongly retarded, lifespan is increased and fertility is greatly reduced (Houthoofd, Braeckman et al. 2002). Although the importance of the microbes for *C. elegans'* fitness was shown in numerous studies the underlying mechanisms for these host-microbe interactions are largely unknown.

C. elegans is a well-established model in many areas of research and recently it has been argued that it also constitutes an excellent model for gaining insights into host-microbiota interactions (Lin and Hackam 2011, Cabreiro, Au et al. 2013). For this field of research *C. elegans* is well suited because it is easily maintained monoxenically in the laboratory and since bacteria represent its food source it can be easily replaced with the bacterial species of choice (Darby 2005). Furthermore the use of a host model with a microbiota that is made up by just one single microbial species has great advantages such as tractability in experimental conditions, especially where both organisms are well suited for extensive genetics studies (Cabreiro, Au et al. 2013).

1.5 Rationale and aims of the study

It is well established that IBD is accompanied by an overshooting of the immune system. Increasingly, it is also thought that alterations in the composition of the microbiota in the intestine contribute to the development of IBD (Mukhopadhya, Hansen et al. 2012). However, very little is known about the specific role of microbiota in the pathogenesis of IBD. Recently it has been suggested that the nematode *C. elegans* might be a suitable model organism for gaining insights into the underlying molecular mechanisms (Cabreiro, Au et al. 2013). The aim of this research project is to evaluate and validate *C. elegans* as new model for investigations into bacterial interplay and host-microbiota interactions and link the results with IBD related studies of microbiota during inflammation and of the immune system. The rationale of the project is therefore to compare and translate findings seen in *C. elegans* to mice and eventually humans. Specifically we are interested in its behavioral and physiological reaction after bacterial exposition. We therefore want to analyze the effects of different bacteria on overall life span and on the production of reactive oxygen species (ROS). It was proposed that *C. elegans* produces ROS in the intestine when exposed to pathogens (Chavez, Mohri-Shiomi et al. 2007). Finally we are hoping to gain insights into possible mechanisms how bacteria interact with the host's immune system by quantifying the effect on prominent immune signaling pathways. Finally we are interested in potential abnormalities in feeding behavior as it has been suggested that *C. elegans* can use aversive olfactory learning to avoid pathogens (Zhang, Lu et al. 2005).

In a first step our aim is to characterize bacterial induced inflammation in *C. elegans* and in a second step to establish a fluorescent based high throughput screening tool for identifying bacterial virulence factors responsible for inflammation processes. Indeed, recently studies have been published that used fluorescence-activated cell sorting (FACS) techniques for automated *C. elegans'* egg and larvae sorting (Fernandez, Mis et al. 2010, Fernandez, Bargmann et al. 2012). We would like to use this method on adult worms. Summarizing our goal is to tackle both sides of the host-pathogen equation within one model organism to find bacterial and host factors that predispose for development of inflammation processes.

2 Material and methods

2.1 NGM plate preparation

NGM plates were prepared according to the wormbook (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html) as follows: For 1 l of NGM medium 3 g NaCl, 17 g agar, and 2.5 g peptone were mixed with 975 ml H_2O and autoclaved for 15 minutes (Autoclave Systec, Tuttnauer). Afterwards the flask was cooled in a 55 °C water bath for 15 minutes. 1 ml 1 M $CaCl_2$, 1 ml 5 mg/ml cholesterol in ethanol, 1 ml 1 M $MgSO_4$ and 25 ml 1 M KPO_4 buffer [108.3 g KH_2PO_4, 35.6 g K_2HPO_4, H_2O to 1 l; pH 6.0] were added. Finally using sterile procedures NGM solution was dispensed into plastic petri plates (diameter 87 mm) using a dispenser (Tecnomat, Integra Biosciences) to fill plates about 2/3 full of agar. Plates were left at room temperature for one day to allow for detection of contaminants, and for excess moisture to evaporate. Plates were stored in plastic bags at 4°C for several weeks.

2.2 Cultivation of bacteria

For bacterial cultivation Lysogeny Broth (LB) or Brain Heart Infusion (BHI) media were used. LB liquid medium was prepared using 10 g/l Tryptone, 5 g/l Yeast extract, 5 g/l NaCl (pH 7.0 +/- 0.2). BHI liquid medium was prepared with 12.5 g/l Brain Heart Infusion solids, 5 g/l Beef Heart Infusion solids, 10 g/l Proteose peptone, 2 g/l Glucose, 5 g/l Sodium Chlorid and 2.5 g/l Di-sodium phosphate (pH 7.4 +/- 0,2). For LB or BHI agar 15 g/l agar were respectively added to the solution. Both media were autoclaved for 20 minutes at 124 °C before use or storage at 4 °C.

E. coli OP50 culture was prepared from a starter culture provided by the CGC from which single colonies were taken and aseptically brought into LB. Inoculated cultures were grown overnight at 37 °C before they were seeded onto NGM plates. For seeding 100 µl of liquid bacteria culture was applied to NGM plates with a pipette (Research Plus; Eppendorf). The drop was spread with a glass spatula all the way to the edges of the plate to secure permanent and reliable exposition. The *E. coli* OP50 lawn was incubated overnight at 37 °C (Function Line Incubator; Thermo Scientific Heraeus) and cooled down to room temperature before worms were added or plates were stored in air-tight plastic bags at 4°C for several weeks (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html).

Similarly all other bacteria strains were aseptically brought into LB or BHI (for further information see Table 2), grown overnight and 100 µl suspension were seeded and spread to the edges of NGM plates. For all bacteria strains streak plates were prepared using an inoculating loop to strike bacteria onto LB of BHI plates. Plates were incubated at 37 °C overnight before they could be stored at 4 °C for several weeks.

Table 2. Defined bacteria strains for lifespan experiment. Listed are strain names, the institutions where they were received from, their suspected inflammatory potential, appropriate culture medium, and incubation time. DSMZ=Deutsche Sammlung von Mikroorganismen und Zellkulturen

Bacteria strain	Source	Inflammatory potential	Culture medium	Incubation time at 37°C
***E. coli* OP50** (gram negative)	MPI Tübingen	Standard/ Baseline	LB	overnight
***E. coli* JM83** (gram negative)	R. Darveau	Pro-inflammatory	LB	overnight
E. coli* JM83 Δ*htrB* +*htrBPg	R. Darveau	Anti-inflammatory	LB	overnight
E. coli* JM83 +*htrBPg	R. Darveau	unknown	LB	overnight
E. coli* JM83 Δ*msbB	R. Darveau	Anti-inflammatory	LB	overnight
***E. coli* mpk** (gram negative)	Microbiolgoy Tübingen	Pro-inflammatory	LB	overnight
***E. coli* Nissle** (gram negative)	Microbiolgoy Tübingen	Anti-inflammatory	LB	overnight
E. faecalis (gram positive)	Prof. Dr. Rudi F. Vogel; TU München	Pro-inflammatory	BHI	overnight
***S.* LT2** (gram negative)	DSMZ	Pro-inflammatory	LB	overnight
S. Dublin (gram negative)	W. Hardt, Schweiz	Pro-inflammatory	LB	overnight
B. vulgatus (gram negative)	Microbiolgoy Tübingen	Anti-inflammatory	Liver in Soy broth	4 days
E. faecium (gram positive)	Microbiolgoy Tübingen	Pro-inflammatory	BHI	overnight
S. epidermidis (gram positive)	Microbiolgoy Tübingen	Pro-inflammatory	LB	overnight
P. aeruginosa (gram negative) ATCC 27853	MiBi Tübingen (Diganostik)	Pro-inflammatory	LB	overnight

One exception to this procedure was used for *Bacteroides vulgatus* for which a liver bouillon was used: liver bouillon was prepared by placing an 1 cm^3 cube of commercially available beef liver in a bouillon of 17 g/l pancreatic digest of casein, 3 g/l enzymatic digest of soya bean, 5 g/l sodium chloride and 2.5 g/l di-potassium hydrogen phosphate. 50 µl of frozen stock solution of *B. vulgatus* was then placed into a 10 ml liver bouillon and anaerobically incubated at 37 °C for two nights. To secure anaerobic conditions air tight containers with an anaerobe package (AnaeroGen 2.5L; Thermo Scientific) were

used. After 48 h 5 ml from the liver bouillon solution were brought into 50 ml BHI liquid medium and incubated anaerobically for further 48 h before it was used for plate preparation. For plate preparation 2 ml of the prepared solution were centrifuged (400 g; 5 minutes) and the pellet was re-suspended in 250 µl of BHI and 100 µl were spread onto NGM plates. Plates were used immediately after preparation and were not stored.
For the experiments the optical density of bacteria culture was determined and standardized. Therefore 100 µl of overnight culture were mixed with 900 µl of Milipore water and the optical density was determined (BioPhotometer, Eppendorf). For comparison 100 µl of pure LB or BHI medium in 900 µl Milipore water was used. The optical density was adjusted to 2 by diluting the bacteria culture with LB or BHI medium.

2.3 *C. elegans* rearing and handling

Experiments were performed with the wild-type *C. elegans* strain N2, originally collected from a mushroom compost near Bristol. The strain for the study was received from the Caenorhabditis genetics center (CGC) in the US. Upon arrival *C. elegans* was bred for several generations on Nematode Growth Medium (NGM) *E. coli* OP50 plates before some worms were frozen using liquid freezing solution for permanent storage. The freezing procedure proposed by the wormbook (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html) was adjusted and used. Worms in different developmental stages were washed off of one or two large NGM plates with 0.6ml S-buffer [129 ml 0.05 M K_2HPO_4, 871 ml 0.05 M KH_2PO_4, 5.85 g NaCl] for each vial to be frozen. Liquid from both plates was collected in a sterile test tube and an equal volume of S-buffer + 30% glycerin (v/v) was added. The mixture was well mixed and 1 ml amounts were aliquotted into 1.8 ml cryovials. The cryovials were packed in an ethanol freezing box for gradual cooling of approximately 1 °C per minute. The box was placed in an -80 °C freezer overnight before the vials were transferred to their permanent freezer locations the next day. One vial was thawed as a tester to check whether the worms survived the freezing procedure. Since their arrival the worms have been maintained in our lab on NGM seeded with *E. coli* OP50 as standard condition at 20 °C (Hybridization Incubator 7601; GFL) in the dark. Animals for all experiments have been maintained under these same housing conditions.

To transfer worms to new plates a worm picker was used. The worm picker was created by connecting a 1-inch piece of platinum wire (32 gauge) to the tip of a Pasture pipet (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html).
Platinum wire conveniently heats and cools quickly and it can be heated often between transfers in order to avoid contamination of the stocks. The end of the wire was flattened slightly with a hammer to avoid harming the worms or poking holes into the agar. To pick a worm identified under the dissecting microscope (Stemi 2000; Zeiss), the flattened tip of the wire was slowly lowered and gently swiped at the side of the worm to lift it up. Long periods off the agar were avoided to prevent the worms from desiccating. To transfer the picked worm onto a new plate, the tip was brought to the surface of the medium, and just left there so that the worm could crawl off the picker onto the agar.

2.4 Lifespan assay of *C. elegans* after bacterial exposure

For lifespan experiments *C. elegans* were age synchronized according to the wormbook (http://www.wormbook.org/chapters/www_strainmaintain/strainmaintain.html): worms were washed off plates with Millipore water and collected in a sterile 15 ml centrifuge tube (Falcon). Millipore water was added to total up to 3.5 ml and 1ml household bleach (5 % solution of sodium hypochlorite) was added. The tube was shaken for a few seconds every 2 minutes. After 10 minutes of incubation the tube was centrifuged (Centrifuge 5810R; Eppendorf) for 1 minute at 2000 rpm for eggs to settle to bottom. Extra water was discarded and replaced by fresh Millipore water. The tube was shaken for a few seconds and centrifuged with the same parameters a second time. This was repeated two times before the eggs were placed onto fresh NGM plates. To prevent overcrowding approximately 10 µl of egg suspension was used per plate. Synchronized L4 *C. elegans* developed in low housing concentrations on *E. coli* OP50 plates.
For the experiment 20 worms were placed onto the bacteria plates of choice. They were transferred to fresh NGM plates seeded with the respective bacteria with a worm picker that was heat sterilized between transferals. For the first 10 days worms were transferred daily. For the subsequent time they were transferred to fresh plates every other day: For every transferal the number of dead *C. elegans* was protocolled. Death was determined upon the lack of movement when touched by the worm picker. Worms that escaped were censored from the experiment. Dead worms found at the plastic edge of the petri dish,

due to unknown reasons, were also excluded from the counting process as this situation was found in all conditions.

2.5 Food choice experiment of *C. elegans* with defined bacteria strains

NGM plates and bacteria cultures in defined optical densities were prepared as described above (see 2.1). For the food choice assay two types of plates were prepared: a two type food choice assay with one defined bacteria strain of choice and *E. coli* OP50 (Figure 3A) and a multi type food choice assay with many defined bacteria strains including *E. coli* OP50 (Figure 3B).

Before the start of the experiment the individual bacterial colonies were selected and incubated overnight with the appropriate medium (Table 2). The following day optical density measurements and seeding onto NGM plates was performed as described previously (2.2). For the food choice assay, plates were designed with a maximal distance between the drops (Figure 3). For both assay types 10 µl drops of bacteria solution were pipetted onto NGM plates. Plates were incubated over night at 37 °C and were used the next day or stored in plastic bags at 4 °C for several weeks and brought to room temperature before experiments. As *B. vulgatus* is strictly anaerobic it was anaerobically cultivated as described above and pipetted onto the plates right before the start of the experiment. Optical density was noted (OD= 33.12) but not standardized.

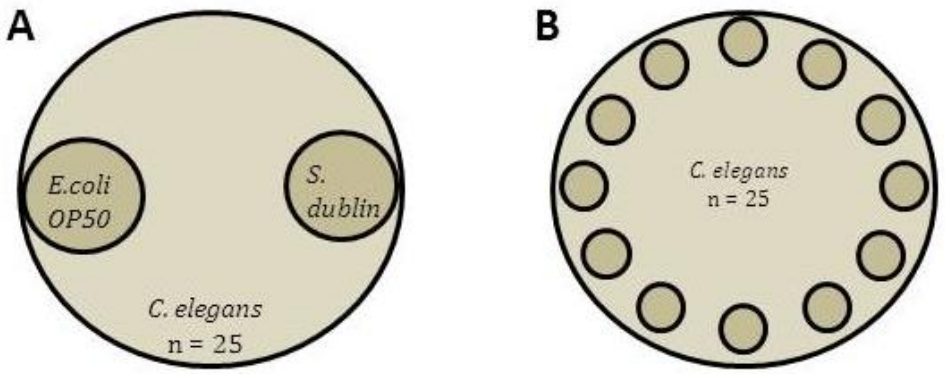

Figure 3. Food choice experimental set up. Light brown background represents NGM plate while dark brown circles represent the bacteria lawns. "*C. elegans*" marks the starting point **A.** shows the set up for a two- bacterial food choice assay while **B.** shows the set up for a multiple bacterial food choice assay where each brown circle represents one bacteria species

Prepared plates with bacteria drops were brought to room temperature and a 10 µl drop of *B. vulgatus* in BHI solution was added to the plate right before the worms were placed

on the plate. Using a worm picker 25 wild type L4-age- synchronized worms were placed onto the plate in equal distance from the bacteria for all food choice assays.
The number of nematodes on each bacteria lawn was determined. Worms with at least half of their body volume on the bacterial lawn were counted. The counting process was performed one, two and four hours after the start of the experiment. Longer periods of time have proven to be dispensable as the food preference situation does not seem to change after four hours.

2.6 Reactive oxygen species (ROS) production in *C. elegans* after defined bacterial exposure

For the reactive oxygen species (ROS) production measurement the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Catalog no. A22188, Invitrogen) was used. The stock and working solutions were prepared according to the instructions delivered with the kit: For the 10 mM Amplex Red reagent stock solution one vial of Amplex Red reagent and one vial of DMSO reagent were brought to room temperature before the Amplex Red reagent was dissolved in 60 µL of DMSO. The 1 reaction buffer was prepared by adding 4 ml of 5 reaction Buffer to 16 ml of Millipore water. For the 10 U/ml Horseradish Peroxidase (HRP) stock solution one vial of HPR was dissolved in 1 ml of the prepared 1 reaction buffer. During the first preparation HRP stock solution was aliquotted into single-use units and store frozen at –20 °C for future use. 20 mM hydrogen peroxide (H_2O_2) working solution was prepared by diluting the delivered H_2O_2 into the appropriate volume of 1 reaction buffer for a final concentration of 3.0 %. The 100 µM Amplex Red reagent and 0.2 U/ml HRP working solution was created by mixing 50 µl of 10 mM Amplex Red reagent stock solution, 100 µl of 10 U/ml HRP stock solution and 4.85 ml of 1 reaction buffer. For quantification a H_2O_2 standard curve was included in the experiment. Thereof the appropriate amount of 20 mM H_2O_2 working solution was diluted into 1 reaction buffer to generate H_2O_2 concentrations of 0 to 10 µM, each in a volume of 50 µl. A non-H_2O_2-containing control was also included.
For the ROS quantification experiments NGM plates and bacteria cultures in defined optical densities were prepared as described above (see 2.2). For the ROS measurement *C. elegans* were allowed to develop on *E. coli* OP50 NGM plates before they were

transferred to defined bacteria conditions at the L4 stadium (Figure 4). The experimental design is depicted in Figure 4 and was made up by two negative control groups one consisting of worms without bacteria exposition and one with exposition to *E.coli* OP50, a positive control group consisting of worms exposed to *E. faecalis* for 18 h, and an experimental group consisting of worms exposed to *E. coli* JM83 for 18 h, 48 h, and 72 h.

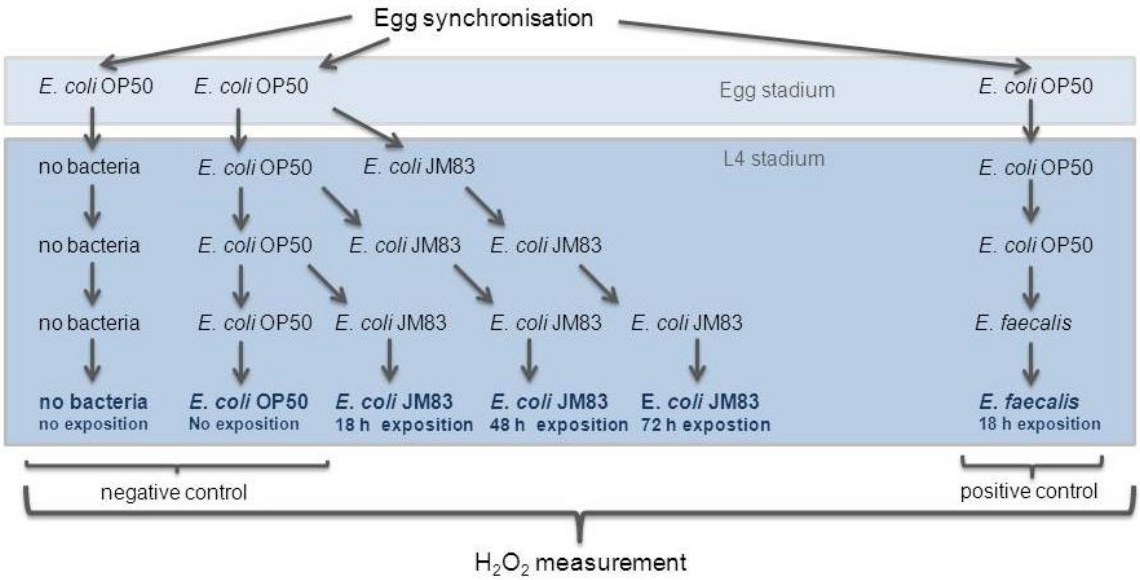

Figure 4. Experimental design for ROS (H_2O_2) quantification. After bleach treatment eggs were allowed to develop on *E.coli* OP50 before adult L4 worms were transferred to the different conditions as indicated in the diagram. Bold, dark blue writing represents time point at which ROS measurement took place.

To secure low housing densities in all conditions for development approximately 10 µl of egg suspension was used per plate. Following L4 worms were transferred to bacteria plates prepared as described under 2.2 and incubated at 20 °C for defined periods of time. The experimental procedures for the hydrogen peroxide measurement were followed according to the protocol by Mohanty and colleagues (Mohanty, Jaffe et al. 1997) and adaptations by Chavez and colleagues (Chavez, Mohri-Shiomi et al. 2007): After exposure to bacteria worms were washed off the plates with 0.25 M sodium phosphate buffer. The buffer was prepared from two stock solutions [stock 1: 2 M NaH_2PO_4 and stock solution 2: 2 M Na_2HPO_4]: 51 ml of stock solution 1 and 49 ml of stock solution 2 were diluted with 100 ml distilled Millipore water and adjusted to pH 6.8. Worms were washed twice in the 0.25 M Sodium Phosphate buffer or until solution was clear. For fluorescent reading (Tecan reader*) C. elegans* were placed in 1 reaction buffer in 0.25 M Sodium Phosphate buffer into a white 96-well plate. Each

well contained a total volume of 100 µl made up by 50 µl of Amplex Red/HRP and 50 µl of worm solution. Each bacterial condition as well as each probe for the standard curve was used in duplicate. Before measurement the plate was incubated in a dark room at room temperature for 30 minutes. For the fluorescent reading the following parameters were used:

Mode:	Multiple reads per well (2x2 (square))
Excitation wavelength:	540nm
Emission wavelength:	590nm
Gain:	54
Number of flashes:	50
Number of cycles:	20
Duration of measurement	10 minutes

2.7 Fluorescence microscopy of superoxid dismutase (SOD) expression in *C. elegans* after exposition with defined bacteria strains

For fluorescence microscopy a reporter worm expressing green fluorescent protein (gfp) under the superoxid dismutase-3 promoter (sod-3::gfp) was used. We received the strain (muls84) from the CGC and proceeded with it as described for the wild-type N2 strain (see 2.1). For fluorescence microscopy live animals were mounted onto agar pads on a glass slide. Agar pads were prepared from a 5 % agar solution in millipore water. A 20 µl drop of liquid agar solution was applied in the middle of the glass slide with a 100 µl pipette tip, with a widened, cut-off end. The glass slide was placed between two glass slides coated with pieces of labeling tape to serve as spacers (Figure 5) (http://www.wormbook.org/chapters/www_intromethodscellbiology/intromethodscellbiology.html). After the drop had been applied it was covered with another clean glass slide on top of the three slides in perpendicular fashion. To flatten the drop the glass slide was gently pressed down. Upon solidification of the agar the taped slides were pulled out and sliding one relative to the other separated the remaining two slides. The agar pad was used right after preparation for it not to dry out.

For mounting of the live worms a 2 µl drop of 15 mM sodium azide (NaN_3) was placed onto the center of the agar pad. NaN_3 was used to anesthetize the worms. 10-20 animals were transferred from NGM plates into the drop using the worm picker as described

above. Following, a coverslip was laid over the animals and the slide was immediately used for microscopy.

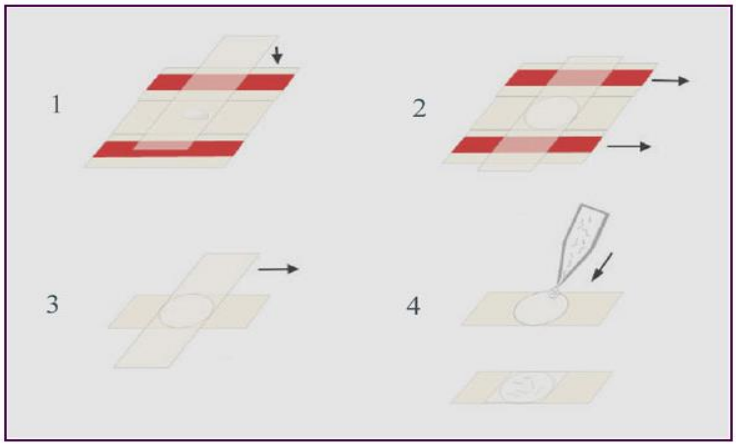

Figure 5. Preparation of agar pads for live mounting of anesthetized animals for fluorescence microscopy imaging (Shaham 2006).

Fluorescence microscopy was performed with a fluorescent microscope (DMRE epi-fluorescent microscope, Leica). Fluorescence images were taken at 2 second exposure time, gain 5x, magnification 5x and gamma 1,29 whereas transmitted light images were taken at 2 milliseconds exposure time, gain 5x, magnification 5x and gamma 1,29. Fluorescent images were quantitatively analyzed using ImageJ (Version 1.48; Wayne Rasband [National Health Institut]) for which the region of interest was selected manually. The user-defined area was measured and pixel values were calculated. Using Excel (Version 2010; Microsoft) averages from duplicates or triplicates were calculated and statistically analyzed using Prims 4 to compare fluorescence levels.

Further fluorescent images were taken on a confocal fluorescence microscope (Zeiss LSM 710 NLO) with identical mounting. In addition to agar mounting Paraformaldehyde (PFA) fixation was used.

2.8 RNA isolation and quantitative PCR of immunoregulatory marker RNA from *C. elegans* after bacterial exposure

To investigate immune system reactions in response to bacterial exposition appropriate primers were needed. The nucleotide sequences for primer of interest were taken from the respective papers or were designed using the primer Blast tool provided by the National Center for Biotechnology Information (NCBI) (see Supplementary Table 1). For design of new primers the whole gene sequence including introns and exons for each gene of interest was extracted from the database on www.wormbook.org. Parameters for primer blast on NCBI were set to PCR product size of 100-400 base pairs, primer melting temperature ranging from 57-63 °C with an optimal temperature at 60 °C, exon-exon junction spanning criteria, and *C. elegans* as reference genome. Proposed primers were back tested with the gene sequence provided by wormbase (https://www.wormbase.org/#01-23-6) for exon-exon junction spanning criteria.

RNA was isolated from synchronized L4 *C. elegans* on NGM plates seeded with bacteria species (compare 2.1). 10 µl of worm eggs solution was placed on the *E. coli* OP50 NGM plates to secure low housing densities for development. In the L4 stadium worms were washed off the plates as described before and 10 µl of worm solution was placed onto NGM with defined bacteria in defined optical densities (see 2.2). *B. vulgatus* NGM plates were prepared similarly to the plates used for the multi type food choice assay (compare 2.4). Instead of pipetting a 10 µl drop of *B. vulgatus* in BHI solution, 100 µl of *B. vulgatus* in BHI were pipetted onto the NGM plates and spread to the edges: NGM plates were brought to room temperature and 100 µl of bacteria solution were added to the plates right before age synchronized L4 worms were placed on the plate. For all bacteria plates *C. elegans* were washed off with nuclease-free water after 24 hours of exposition time, and then washed 2-3 times or until the water was clear of visible bacteria remains. Next 75 µl of worm solution from one bacterial condition was placed in Eppendorf tubes and shock frozen in liquid nitrogen for 15 seconds. For worm lysis 500 µl Trizol was added and incubated for 10 minutes at room temperature. Following 140 µl chloroform was added and vortexed for 15 seconds to secure a homogeneous mixture. The solution was incubated for 2 minutes at room temperature and centrifuged (Centrifuge 5417R, Eppendorf) for 15 minutes at 4 °C. The clear phase was transferred into a fresh Eppendorf tube and filled up with an equal amount of

70 % ethanol to a total volume of approximately 700 µl. For the RNA extraction the RNeasy Mini Kit (Qiagen, Catalogue no. 74106) was used according to manufacturer's instructions.

The RNA quality was then analyzed using a spectrophotometer (Nanophotometer, IMPLEN). Analyses were used to determine the RNA purity and the RNA concentration for subsequent DNA digestion. RNA purity can be estimated by the ratio between the absorbance values at 260 nm and 280 nm. According to the manual a ratio of ~2.0 can be accepted as "pure" for RNA. For our experiments ratios ranging from 2.0-2.2 were measured and used. Another essential criterion for appropriate RNA quality was an absorption curve with two peaks as measured by the spectrophotometer. For quantification the spectrophotometer was blanked with 3 µl nuclease-free water before 3 µl of the eluted RNA was measured with the following parameters:

Nucleid acid type	dsRNA
Lid-Factor	50
Dilution Factor	1000
Factor	40.0
Units	µl /ml

The RNA was used directly or stored at -80 °C for several months. For primer testing RNA was cleared from DNA digestion and transcribed into cDNA using the DNA-free DNA Removal Kit (Cat No. AM1906, Life technologies) and QuantiTect Reverse Transcription Kit (Cat No. 205313, Qiagen). The kits were used according to their instructions: For DNA digestion 5 µg of RNA was needed. The appropriate volume containing 5 µg of RNA was determined after photometric RNA quantification and totaled up to a volume of 50 µl with nuclease-free water. It was then incubated with 5 µl 10x DNAse buffer and 1 µl of rRNAsin at room temperature. After 30 minutes of incubation 6 µl of DNAse inactivation solution was added to stop the reaction and incubated for 2 minutes at room temperature. Finally it was vortexed a few times over the course of 2 minutes and the clear, RNA-containing, fraction was transferred to a fresh RNA-free tube. The RNA was stored at -80 °C for several months. After DNA digestion the RNA was transcribed into cDNA. 14 µl of template RNA were mixed together with 4 µl Reverse Transcriptase Buffer (5x), 1 µl Reverse Transcriptase Primer Mix, and 1 µl

of the enzyme Reverse Transcriptase. The tube was incubated at 42 °C for 30 minutes before the reaction was stopped by incubation at 95 °C for 3 minutes.

For the PCR mix following components and quantities were used per sample:

2.5 µl	10x buffer
0.5 µl	10 mM DNTPs
2 µl	$MgCl_2$
15. 8µl	nuclease-free water
0.2 µl	Taq Polymerase
1.5 µl	forward primer (primer concentration 10 µM)
1.5 µl	reverse primer (primer concentration 10 µM)
1 µl	template cDNA

The PCR reactions were realized with a thermocycler (C1000 Touch, Biorad) with these reaction conditions: 94 °C for two minutes, followed by 35 cycles of 94 °C for one minute, 60 °C for one minute, and 72 °C for 35 seconds, as well as five final minutes at 72 °C. Finally, the reaction was finished by cooling to 4 °C.

PCR products were then separated by electrophoresis on a 2 % agarose gel made up with standard Tris-Borate-EDTA (TBE) 0.5x buffer. Depending on the number of samples combs with 15 or 30 wells were used. For each primer pair a sample with (positive control) and without (negative control) cDNA was run. All PCR products were run on an agarose gel (see Supplementary Figure 1). Positive controls were included to check whether the desired amplicon length was amplified, while negative control samples were used to test for contamination. All primer pairs listed in Supplementary Table 1 were checked for specificity by gel electrophoresis.

For electrophoresis wells were filled with 10 µl DNA/PCR mix and 5 µl loading buffer. 5 µl of Gene Ruler 1 kp DNA Ladder (Cat No. SM0313, Life technologies) was used as a size marker on all electrophoreses. The gel ran on average for 45 to 60 minutes at 120 V (Power Pac 200, Biorad) and was then stained in an ethidium-bromid water bath for 10 minutes and washed in a water bath for 5 minutes. Afterwards it was placed into a fluorescent chamber (Ti5, Biometra) for visualization of ethidium-bromide under UV light. For photographic capture, adjusting exposure time optimized brightness and best results were usually achieved at exposure times between 1 and 2.5 seconds.

Images of the electrophoresis gels are shown for primer pairs that yielded the desired amplicon length Supplementary Table 2. For these primer pairs lightcylcer analyses with DNAase-digested RNA were performed to test amplification efficiency.

Pooled RNA extracted from different samples for which up regulation of the respective mRNA could be expected was used as a standard; pooled RNA extracted from samples for which down regulation could be expected was used as a mock. Samples without RNA were also included for each primer. For the standard the pooled RNA was used undiluted as well as in following dilutions: 1:5, 1:25, and 1:125. For the reaction the kit QuantiFast SYBR Green RT-PCR was used. This is a one-step real time RT-PCR system that combines the reverse transcriptase and the PCR step. It was prepared according to its instructions: A reaction mix made up by 5 µl 2x QuantiFast SYBR Green RT-PCR Master Mix, 0.1 µl forward and 0.1 µl reverse primer (primer concentration 100 µM), 3.7 µl nuclease-free water, 0.1 µl QuantiFast RT Mix as well 1 µl of RNA (standard or mock) or nuclease-free water (RNA-free sample) per well was prepared. The 2x QuantiFast SYBR Green RT-PCR Master Mix contains HotStarTaq Plus DNA Polymerase, a polymerase that is inactive at ambient temperature, QuantiFast SYBR Green RT PCR Buffer, the fluorescent component SYPR Green l, and ROX passive reference dye as baseline fluorescence levels for normalization. The QuantiFast RT Mix contains Omniscript and Sensiscript blend, which are enzymes facilitating the transcription process. The appropriate volumes were dispensed into a 96 well plate, which was kept on ice for the entire pipetting process. 1µl of template RNA or RNA-free water was added into the corresponding wells. The plate was placed in the RT-PCR cycler (Lightcycler 400 II, Roche) and run with following parameter:

Reverse Transcriptase	1 cycle	10 minutes	50 °C
Denaturation	1 cycle	5 minutes	95 °C
PCR Quantification	40 cycles	10 seconds	95 °C
		30 seconds	60 °C
Melting Curves	1 cycle	5 seconds	95 °C
		1 second	46 °C
		1 second	95 °C
Cool down	1 cycle	20 seconds	40 °C

For each primer pair light cycler data was interpreted using LightCycler® 480 1.5.0 SP3 (Version 1.5.39; Roche), which generated amplification curves, for which fluorescence levels are plotted against the number of cycles, as well as a standard curve, and melting

curve. The standard curve was generated from all standards of one primer pair by plotting the melting temperature against the log of the template amount. The Melting curves were created by plotting the measured fluorescence against the temperature resulting in curves with peaks at their respective melting temperature. For interpretation of the data the efficiencies and respective errors were analyzed. Curves with peaks at lower melting temperatures than the melting curves of the specific PCR products suggest the formation of primer-dimers, while multiple peaks at different melting temperatures or plateaus indicate formation of nonspecific products or smear (SYBR green handbook). Primer that showed increasing Cp values with increasing dilutions, a high melting temperature for the negative sample, desirable efficiency values and standard errors were used for further experimental analysis of mRNA expression.

Table 3 shows all primers that were used for final qPCR analysis and the respective lightcylcer parameters for these primers can be found in the supplementary Table 2.

Table 3: Primer pairs used for qPCR analysis. These primer pairs were tested in PCR and gel electrophoresis and yielded the expected gene length. Numbers in front of the gene name indicate that multiple primer pairs were tested. "F" or "r" at the end of the gene name indicate forward and reverse primer. The Table shows the primer sequences, where the sequence was obtained from, and the amplicon size.

Gene	Primer Sequence	Source	Amplicon Size
act-1_f	**GCTGGACGTGATCTTACTGATTACC**	**(Hoogewijs, Houthoofd et al. 2008)**	**114**
act-1_r	**GTAGCAGAGCTTCTCCTTGATGTC**		
ama-1_f	**CCTACGATCTATCGAGGCAAA**	**(Hoogewijs, Houthoofd et al. 2008)**	**129**
ama-1_r	**CCTCCCTCCGGTGTAATAATG**		
daf-16_f	**TTTCCGTCCCCGAACTCAA**	**Zhang, Jie et al. 2009**	**116**
daf-16_r	**ATTCGCCAACCCATGATGG**		
4_lys-7_f	**TGTCTCCAGAGCCAGACAATC**	**NCBI Primer Blast**	**136**
4_lys-7_r	**TCCACCGCTGTACACATTCC**		
1_spp-1_f	**CTCTCGTCGAGGGTGGAGA**	**NCBI Primer Blast**	**197**
1_spp-1_r	**ATCAACATCCTTGCACGCCT**		
1_tol-1_f	**GGATAGTGGGATGCGAGACC**	**NCBI Primer Blast**	**220**
1_tol-1_r	**TGTAAATGCGGCAGAGAGCA**		
1_pmk-1_f	**TCGCCGTGATTTCAAACGTC**	**NCBI Primer Blast**	**267**
1_pmk-1_r	**GCAAATGCCACGTTTTTCTGGA**		

The data was further analyzed using the lightcycler software to determine mRNA expression levels. The data was interpreted by the Ct values which mark the cycle in which the fluorescent level corresponding to an exponential amplification can first be observed. The Ct values were used for mRNA quantification. Additionally as internal

control to secure sole amplification of the desired gene sequences melting temperature were checked. Melting temperatures mark the temperature at which the DNA denatures so that the SybrGreen is released and following the fluorescent level declines rapidly. Melting temperatures depend on the amplicon length and the C-G content and are highly specific for each fragment. Congruent peaks of different melting curves represent the same amplified amplicon and a specific qPCR. Conversely if more than one peak had been found for the same RNA sample the data would have been discarded as multiple DNA-fragments would have been amplified simultaneously. For mRNA quantification the Ct values of the gene of interest were tested against the Ct value of the housekeeping gene. For the calculations of relative differences in transcription the following formula was used:

$$R = \frac{E_{Testgene}^{Ct(sampleA)_{Testgene} - Ct(sampleB)_{Testgene}}}{E_{Housekeepinggene}^{Ct(sampleA)_{Housekeepinggene} - Ct(sampleB)_{Housekeepinggene}}}$$

Sample A is the standard for which mRNA of *C. elegans* exposed the *E. coli* OP50 was used. Sample B is mRNA of C. *elegans* exposed to defined bacteria species of choice. The ratio (R) shows relative differences in gene expression of *C. elegans* exposed to bacteria of interest compared to *E. coli* OP50 normalized to actin expression respectively. E represents the efficiency for each primer and is the slope of a curve calculated from the four standard values (pooled mRNA and dilutions in 1:5; 1:25; 1:125). An efficiency of two would be ideal but efficiencies between two and three were accepted and used for the following analyses. Whenever leaving out one of the four standard values resulted in better efficiency values this procedure was used.

2.9 Statistics

For all statistical analysis and graphs Prims 4 (Version 4.03; GraphPad Software Data) was used. Before statistical analysis data for each trait was checked for normality use the Shapiro-Wilk normality test. If normality was not given for one parameter or for all groups the oneway Kruskal-Wallis Anova and Dunn's multiple comparison test was used for comparison of multiple groups. For analyses of the survivorship curves the log rank test was used.

3 Results

3.1 Determination of *C. elegans* survival rates after exposure to different bacterial strains

As bacteria play a role in host fitness (Zilber-Rosenberg and Rosenberg 2008) we wanted to assess the contributions of the different bacteria on overall lifespan. Bacterial strains for exposure of *C. elegans* were chosen according to their pro- or anti-inflammatory potential (Tab. 2).

The *E. coli* JM83 strain and its mutants were obtained from the Darveau lab (Bainbridge, Coats et al. 2006) and will be introduced briefly here. Lipopolysaccharide (LPS) in the outer membrane of gram-negative bacteria is a potent toxin. Its biological activity is largely determined in the lipid A region by differences in number, length and placement of the fatty acids and phosphate groups: fewer fatty acids or phosphate groups are associated with lower biological activity of the lipid A structure (Rietschel, Kirikae et al. 1994). In *E. coli* several transferases, including HtrB and MsbB, catalyze the incorporation of laurate (HtrB) and myristate (MsbB) into lipid A (Clementz, Bednarski et al. 1996, Bainbridge, Coats et al. 2006). *Porphyromonas gingivalis* (gram- negative) has a LPS structure with an underacylated, underphosphorylated lipid A that contains long-chain and branched fatty acids and is characterized by a reduced ability to stimulate host responses (Darveau, Cunningham et al. 1995). The *E. coli* JM83Δ*htrB+htrBPg* mutant expresses the HtrB homologue of *P. gingivalis* (HtrBPg) which results in a lipid A with palmitate (C16) at the position normally occupied by laurate (C12) in the wild type (Bainbridge, Coats et al. 2006). In the *E. coli* JM83 *+htrBPg* mutants some lipid A are with laurate and others with plamitate. The *E. coli* JM83 Δ*msbB* mutant lacks a secondary myristate chain of lipid A: for the *E. coli msbB* null mutant that is lacking the secondary myristate a 10.000-fold lower ability to induce inflammation as measured by TNFα induction (Darveau, Cunningham et al. 1995).

For this and all following experiments appropriate amplification conditions of bacteria were identified and used: Bacterial strains were kept and stored on LB agar plates as described in the material and methods section. Before the start of the experiment the individual bacteria colonies were selected and incubated overnight with the appropriate

medium as described above (Table 2). The following day optical density measurements and seeding onto NGM plates was performed (see 2.2).

For all following experiments described under 3.1. age-synchronized, wild-type *C. elegans* at densities of 20 worms per plate were performed in duplicate. Eggs were maintained on NGM plates seeded with *E.coli* OP50 until they reached the L4 developmental stage. For the experiment the worms were transferred to freshly prepared NGM plates seeded with the defined bacterial strains at equal optical density with a worm picker. Observations from pre-experiments indicated that bacteria densities also mediate an effect; e.g. whereas low densities of the food source bacteria *E. coli* OP50 are beneficial for survival, higher bacterial numbers seem to have increasing detrimental side effects. Using bacteria in defined optical densities is an important novel adaptation to existing protocols found in the literature and this standardization was used in all following experiments.

To assess the effect of pro- and anti-inflammatory bacteria on the worm's survival rates we analyzed overall lifespan. As shown in figure 6 the survival rate of *C. elegans* upon life time exposure to different bacteria strains is illustrated in dependence of the survival day. In the lifespan assay there was a detectable overall difference between bacterial strains ($p<0.01$; log-rank test). Compared to *E. coli* OP50 worms feeding on *S.* Dublin ($p<0.01$; log-rank test) and *S.* LT2 ($p=0.07$; log-rank test) had a shorter life span (Figure 6). When *C. elegans* were feeding on *E. coli* Nissle, *E. coli* mpk, *E. coli* JM83, and *E. faecalis* compared to feeding on *E. coli* OP50 there was no statistical difference (Figure 6).

Figure 7 shows the survival rate of *C. elegans* upon life time exposure to different bacteria strains in dependence of the survival day. In the lifespan assay there was a detectable overall difference between bacteria strains ($p<0.01$; log-rank test). Compared to *E. coli* OP50 worms feeding on *E. faecium* had a longer life span ($p<0.01$; log-rank test; Figure 7). For *C. elegans* feeding on *E. coli* JM83 +*htrBpg*, *E. coli* JM83 Δ*htr+BhtrBPg*, and *S. epidermidis* compared to feeding on *E. coli* OP50 there was no statistically significant difference (Figure 7).

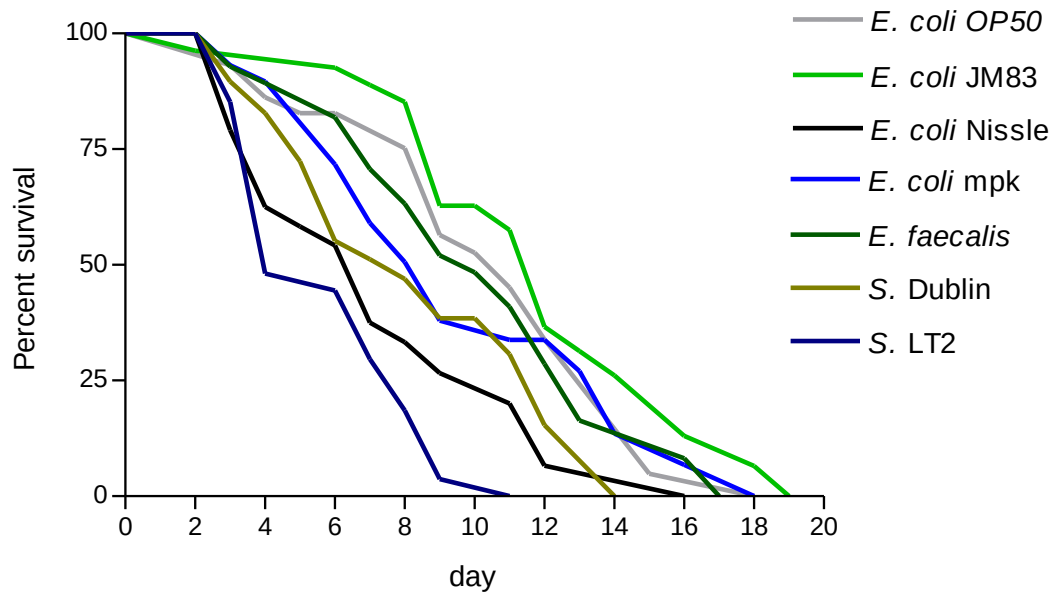

Figure 6. Effect of different bacteria strains on lifespan of *C. elegans*. The graph shows survival curves for wild type *C. elegans* exposed to different bacteria (shown in different colours). Median lifespan in days: on *E. coli* OP50=11; on *E. coli* JM83=12; on *E. coli* Nissle=7 on *E. coli* mpk=9; on *E. faecalis*=10, on *S.* Dublin=8, and on *S.* LT2=4; there is an overall statistical significant difference (p< 0,01; log-rank test). Number of worms per condition n= 40.

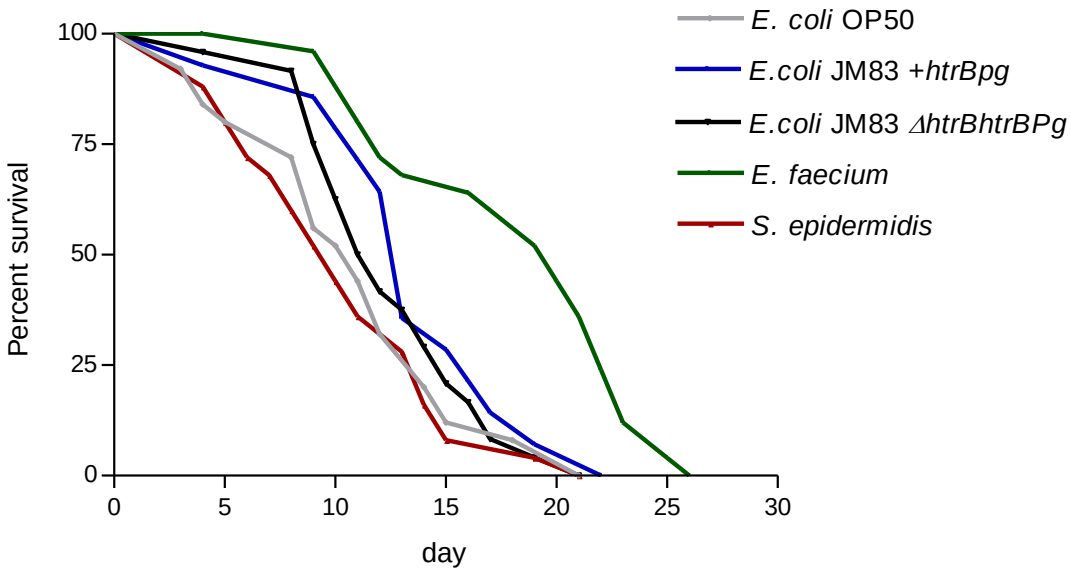

Figure 7. Effect of different bacteria strains on lifespan of *C. elegans*. The graph shows survival curves for wild type *C. elegans* exposed to different bacteria (shown in different colours). Median lifespan in days: on *E. coli* OP50=11; on *E. coli* JM83 +htrBpg =13; on *E. coli* JM83 ΔhtrB+htrBPg = 11.5; on *E. faecium*=20; on *S. epidermidis*=10; there is an overall statistical significant difference (p< 0,01; log-rank test). Number of worms per condition n= 40.

3.2 Analysis of *C. elegans'* food choice preference for defined bacteria

C. elegans can use aversive olfactory learning to avoid pathogens, which has been described as a form of adaptive immunity (Zhang, Lu et al. 2005) .We therefore wanted to assess its avoidance behavior when presented with different bacteria strains. All bacterial strains from Table 2 were tested for their induction potential of aversive olfactory learning in *C. elegans*. Food choice plates were prepared as described under 2.5. Figure 8 shows the ratio of worms on a given bacteria species compared to worms on *E. coli* OP50 in a two choice comparison, e.g. there was a choice between *E. coli* OP50 and one other bacterial species. The line marks the ratio for indifference between *E. coli* OP50 and the bacteria of choice. As the value for *E. coli* OP50 was used as baseline and therefore incorporated in the ratio calculations a statistical test would not be valid. However, for *E. coli* mpk, *E. faecalis*, *E. faecium*, and *S. epidermidis* the respective mean and standard deviation did not contain a ratio of one pointing to significant difference in feeding preference.

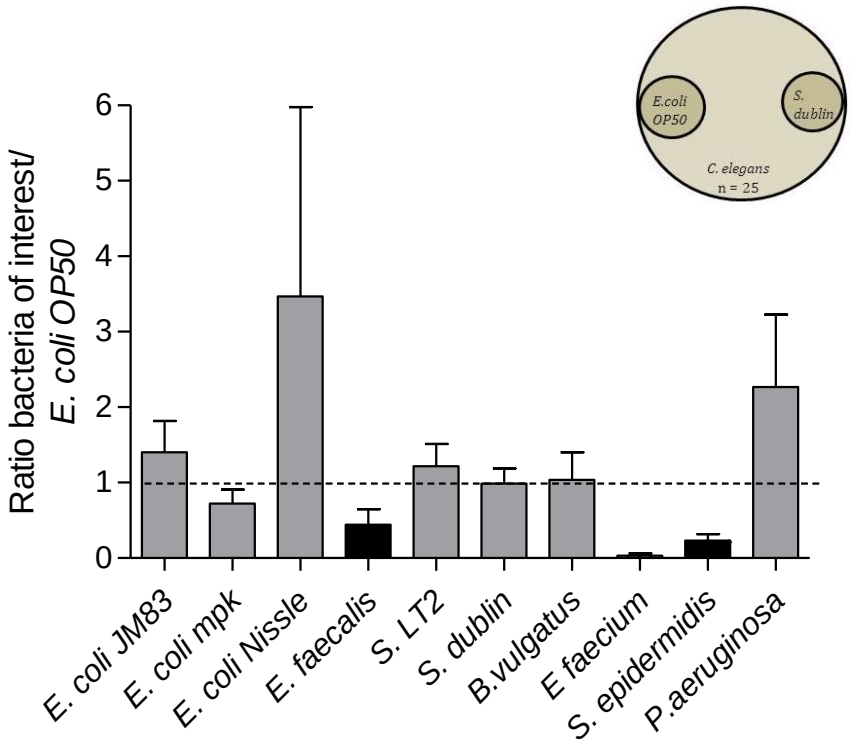

Figure 8. Results of feeding assay. For experimental set up see Figure 3A. Graph shows ratios; ratio of 1 means equal number of animals on both bacteria lawns. Worm counts were performed after four hours of exposition time.Grey bars represent gram negative, black bars gram positive bacteria strains. Error bars represent standard deviation (SD). n= 25 animals per plate, four plates per condition; overall n = 100 per bacteria condition.

Figure 9 shows the percentage of worms on a given bacterial species in a multiple choice comparison, e.g. there was a choice between *E. coli* OP50 and all indicated bacterial species. Since data was not normally distributed in all groups as analyzed by the Shapiro-Wilk normality test a one-way Kruskal-Wallis ANOVA was performed. It indicated an overall statistical significance and further analysis revealed that there are statistically significantly less worms on *E. faecalis* ($p<0.01$; Dunn's multiple comparison test) and *E. faecium* ($p<0.01$; Dunn's multiple comparison test) than on *E.coli* OP50. Further comparisons to feeding on *E.coli* OP50 by Dunn's multiple comparison test were not statistically significant.

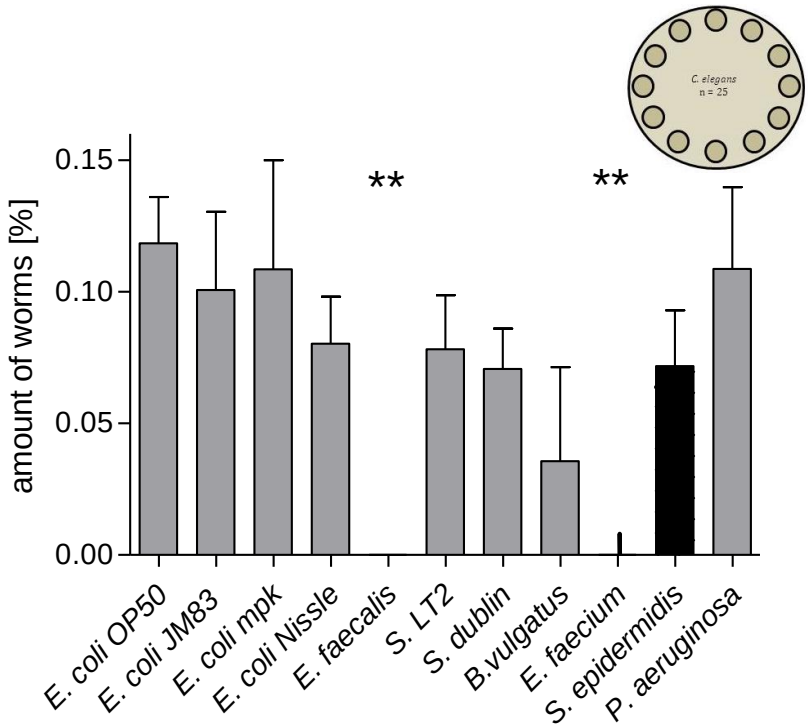

Figure 9. Results of feeding assay. For experimental set up see Figure 3B. Graph shows percentages. Worm counts were performed after four hours of exposition time. Grey bars represent gram negative, black bars gram positive bacteria strains. There is a statistical significant overall difference as measured by a Kruskal-Wallis one-way ANOVA ($p<0.001$; $n=7$). Stars indicate statistically significant differences to *E.coli OP50* as analyzed by the Dunn's multiple comparison test. Error bars represent SD. n= 25 animals per plate, seven plates per condition; overall n= 175.

3.3 Analysis of Reactive Oxygen Species (ROS) as a marker for inflammation in *C. elegans* after defined bacteria exposition

Hydrogen peroxide is one of the by-products of an oxidative burst as part of a defense mechanism against pathogenic bacteria in many species (Cross and Segal 2004). The Amplex Red assay can be used to detect peroxidase released from biological samples including cells (Zhou, Diwu et al. 1997, Song, Al-Mehdi et al. 2001, Votyakova and

Reynolds 2001). Chavez et al. adapted this assay for peroxidase detection in *C. elegans* (Chavez, Mohri-Shiomi et al. 2007). We used the assay to investigate the hydrogen peroxide release from whole worms after different exposure times to defined bacterial species (Figure 4). For analysis different numbers of *C. elegans* per well were used (n= 20; n= 30; n=50; n=100). Numbers were determined by counting the number of worms in a 2 µl drop of reaction buffer worm solution. The adequate amount of solution was then added to the well and filled up with reaction buffer to a total of 50 µL. Control wells with reaction buffer without worms were included in the analysis. Worms were exposed to the defined bacteria for different lengths of time: L4-worms transferred to NGM plates without bacteria or with *E.coli* OP50 used as negative controls. A positive control was set up with *C. elegans* exposed to *E. faecalis* for 18 h, as it was shown to have elevated peroxide production compared to exposure to *E. coli* OP50 (Chavez, Mohri-Shiomi et al. 2007). The experimental group was made up by *C. elegans* exposed to *E. coli* JM83 for 18 h, 48 h and 72 h (Figure 4).

Each condition was performed in duplicate including the probes for the standard curve. Amplex Red/HRP solution was added and the plate was incubated as described above. The fluorescence reading was performed as indicated in 2.6.

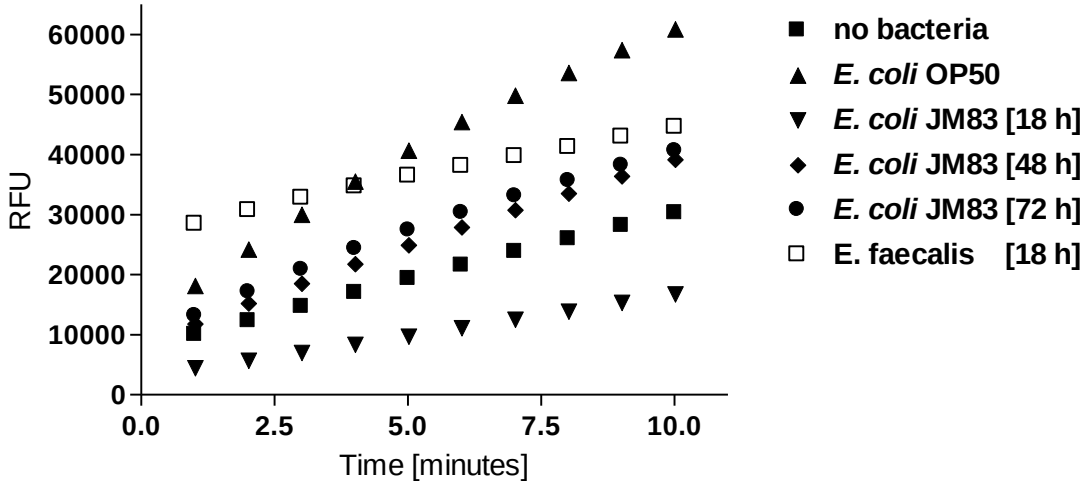

Figure 10. Effects of bacterial exposition on hydrogen peroxide production in *C. elegans*. Different symbols indicate exposition to different bacteria species and for different exposition times (as indicated in the legend). X-axis shows relative fluorescence units (RFU) as measured by the fluorescent reader as parameter for hydrogen peroxide production in *C. elegans*, y-axis shows the time in minutes. Maxima for all bacterial conditions are found at the latest measurement point: Each measurement point is averaged from two samples. Kruskal-Wallis one-way ANOVA did not show statistical significant differences at maximum RFU. n= 20; number of worms per well.

Figure 10 shows the course of the fluorescent reading as indicator for ROS production after bacterial exposition for exposure time as indicated. Comparing RFUs at curve maxima revealed that worms exposed to *E. coli* JM83 for 18 hours or without bacterial exposition compared to worms feeding on *E.coli* OP50 had significantly less ROS production. However Kruskal–Wallis one-way ANOVA did not show any statistical significant differences probably due to the small sample size of two samples per point.

3.4 Analysis of Superoxid Dismutase-3 as a marker for inflammation induction in *C. elegans* after exposition to different bacteria

3.4.1 Analysis SOD expression in exposed *C. elegans* by fluorescence microscopy

Some bacteria kill *C. elegans* by extracellular ROS production (Jansen, Bolm et al. 2002). In response *C. elegans* upregulates enzymes such as superoxide dismutase that catalyze the removal of O_2^- thereby protecting against oxidative stress (Honda and Honda 1999).
Animals were washed off from *E. coli* OP50 NGM plates and transferred to bacteria plates of choice when they had reached the L4 adult stage. After exposition periods as stated they were prepared for microscopy (see 2.7). Microscopy was performed on a fluorescent microscope (DMRE epi-fluorescent microscope, Leica) using parameters as indicated in section 2.7.
For better visualization of the location of the fluorescence within the animals light microscopy images were taken in addition to every fluorescent image. Using Photoshop CS (Version 8.0.1; Adobe) these two images were merged. An example is shown for *C. elegans* exposed to *E. coli* OP50 compared to *C. elegans* exposed to *E. faecalis* (Figure 11).
Fluorescent images were quantitatively analyzed using ImageJ and Prism 5 as described in section 2.7. to compare fluorescence levels of SOD-3 induction in *C. elegans* after 24 hours exposition period on different bacterial species. Fluorescent images that were used for quantification can be found in Supplementary Figure 2.

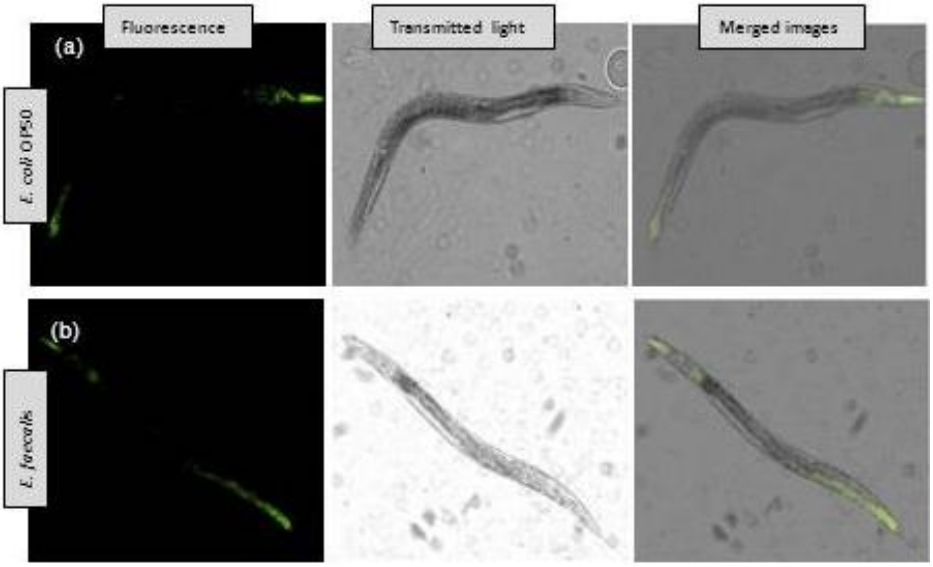

Figure 11. Fluorescence and light microscopy of reporter *C. elegans.*
Fluorescent microscopy was performed after exposure to different bacteria strains. SOD-3::gfp worms in the L4 stadium were exposed to *E. coli* OP50 (a) or *E. faecalis* (b) for 24 hours. Images were taken to confirm SOD-3 expression in head and tail. Fluorescent and light microscopy images were taken and merged. (Microscopy magnification 5x)

A Kruskal-Wallis one-way ANOVA indicated no overall statistical significance probably due to the small sample size of two worms per condition. However, the graph shows that *S.* LT2 and *S.* Dublin have more SOD-3 induction than worms feeding on *E.coli OP50* (Figure 12).

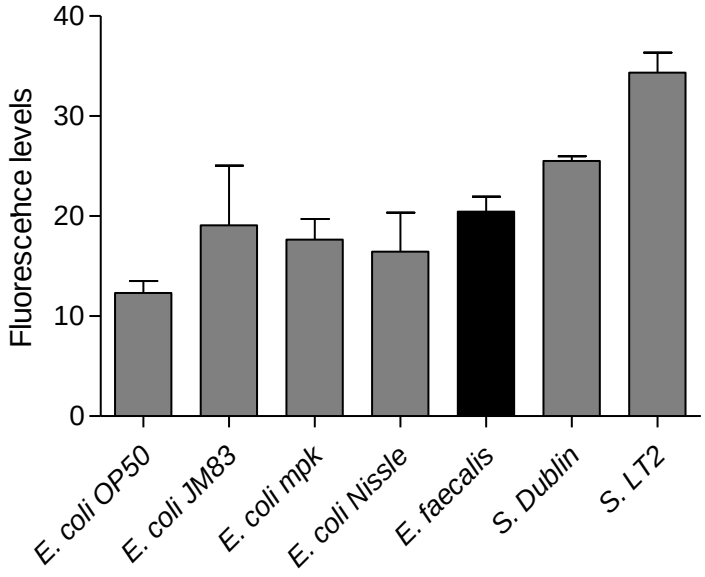

Figure 12. Analysis of fluorescence levels as marker for SOD-3 expression in sod-3::gfp worms. Fluorescent images are shown in Supplementary Figure 2. Images were analyzed by ImageJ freeware. For each exposition condition two worms were analyzed, except for *E. coli OP50* for which 3 worms were analyzed. There is no statistical significant difference as a measured by A Kruskal-Wallis one-way ANOVA. Error bars represent SEM.

3.4.2 Visualisation of SOD expression in exposed *C. elegans* by confocal fluorescence microscopy

Confocal microscopy offers various advantages over conventional microscopy, including extremely high-quality images, much higher resolution of structures, and reduction of background information. We therefore wanted to assess theses potential benefits by visualizing *sod-3::gfp* by confocal fluorescent microscopy imaging.

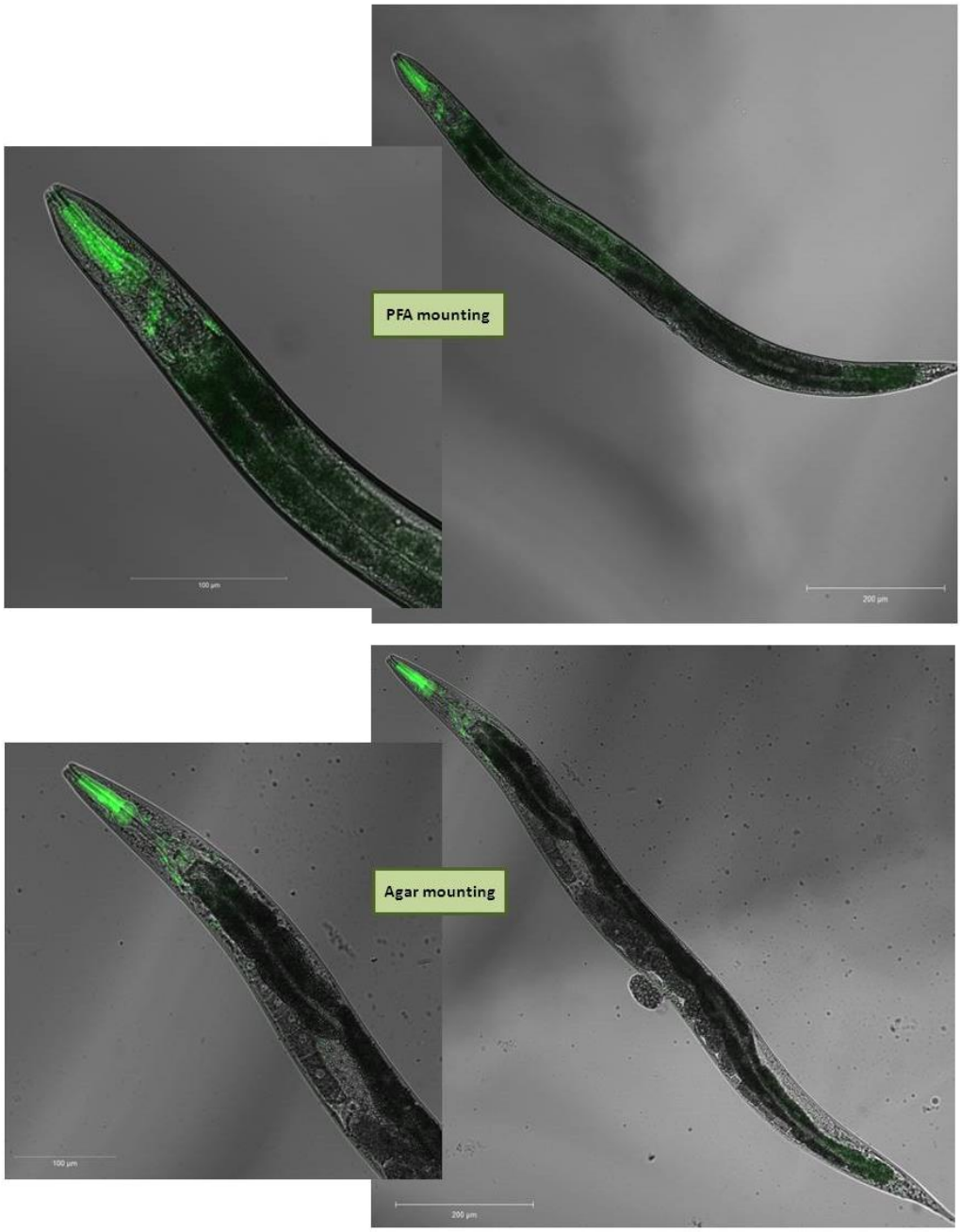

Figure 13. Confocal fluorescence Microscopy of reporter *C. elegans* mounted in agar and PFA. Images were taken after exposure *to E. coli OP50 for 24hours*. SOD-3::gfp worms in the L4 stadium. Worms were mounted using PFA or agar mounting as indicated in the images. Fluorescent images were taken with a confocal fluorescent microscope, magnification 10x and 20x. Size according to scale bar.

Animals were prepared like for conventional fluorescent microscopy (described in section 2.7). Microscopy was performed on a confocal fluorescent microscope (Zeiss LSM 710 NLO). Various images were taken but fluorescence with reporter worms after exposition to *E. coli* OP50 for 24 hours. Animals were mounted in agar as well as fixed in PFA. Differing parameters were tested and various images were taken. The display in Figure 13 shows exemplary images taken by the confocal fluorescent microscope for PFA and agar mounting. In a similar manner to fluorescent imaging described under 2.7 further images were taken on the confocal microscope after *C. elegans* exposition to various bacteria strains. However not enough images were taken to perform statistical analysis.

3.5 Analysis of immunoregulatory marker RNA in *C. elegans* after exposition to defined bacteria

Whether immune response gene regulation may be responsible for protection or for inflammation induced dysregulation relevant immune parameters were analyzed. To investigate the role of the toll dependent MAP Kinase pathway during bacterial infection *tol-1* and *pmk-1* expression was analyzed during infection by qPCR. The expression pattern of *daf-16* was also studied as *daf-16* is another important immune pathway transcription factor in *C. elegans* against infection by many bacterial species. Furthermore two late response genes: *lys-7* and *spp-1* were examined.

Three qPCR runs were performed as described above (see 2.8) so each primer and bacterial condition was at least run in triplicate. Primers were used to target the genes *pmk-1, lys-7, spp-1, tol-1, daf-16* (compare Table 1) as well as act-1 (housekeeping gene) and the same bacterial conditions were selected as for the other experiments. Ct values that were extrapolated from the standard curve by the LightCycler software were discarded from the data analysis. Likewise Ct values that came within the limits of the last 5 cycles of the run were excluded from analysis as they bear a very high uncertainty. Altogether a minimum of one and maximum of four ratios was calculated for each gene. A ratio of one represents no difference in mRNA expression when exposed to bacteria of interest compared to *E. coli OP50* whereas a ratio of 1.5 means a 1.5 fold higher gene expression when exposed to the respective bacterium compared to *E.coli OP50*. As all baseline values are incorporated in the value calculations (compare formula under 2.8) a statistical test would not be valid. Arguing from a biological point

of view a 1.5 fold elevation or 0.5 fold reduction in mRNA expression seems to point to a relevant difference in cell signaling. Therefore means and their respective SD that were 0.5 or lower and accordingly 1.5 or higher are treated as significant. Cases with only one underlying biological sample are excluded from this procedure. According to these criteria worms exposed to *E. coli* JM83, *E. coli* JM83 Δ*msb*, *E. coli* Nissle, *E. faecalis*, and *E. faecium* had significantly lower *pmk-1* expression compared to exposition to *E. coli OP50* (Figure 14A). As seen in Figure 14B lysozyme 7 (*lys-7*) mRNA expression was significantly lower in *C. elegans* exposed to *E. coli* JM83, *E. coli* Nissle, *E. faecalis*, *S.* LT2, *S.* Dublin, *S. epidermidis*, and *P. aeruginosa*. Furthermore there was significantly lower *spp-1* expression when worms were exposed to *E. coli* JM83, *E. coli* JM83 +*htrBPg*, *E. faecalis* and *P. aeruginosa* (compare Figure 14C). Compared to exposition of *C. elegans* to *E. coli* OP50 there was significantly less *tol-1* expression after exposition to *E. coli* JM83, *E. coli* Nissle, *E. faecalis* and *S. LT2* (see Figure 14D). The qPCR data for *daf-16* is less reliable as fewer replicates are present for most bacteria conditions but as seen in Figure 14E there is significantly less *tol-1* expression at exposure to *S.* LT2 than to *E. coli* OP50 and by trend there is less *tol-1* expression at exposure to *E. coli* JM83 Δ*msb*, *E. coli JM83* Δ*htrB*+*htrBPg* and *S.* Dublin for all of which only two valid replicates are existent. Altogether exposition to *E. coli* JM83 compared to *E. coli* OP50 resulted in less mRNA expression for all genes tested, exposition to *E. faecalis* in less expression of all genes except *daf-16*, and exposition to *E. coli* Nissle to less expression of *pmk-1, lys-7* and *tol-1*. Summarizing by trend it is shown that *C. elegans* exposure to different bacteria species resulted in equal or lower mRNA expression of *pmk-1, lys-7, spp-1, tol-1,* and *daf-16*. It is noticeable that exposure to *E. coli* JM83 resulted in lower mRNA expression of all genes.

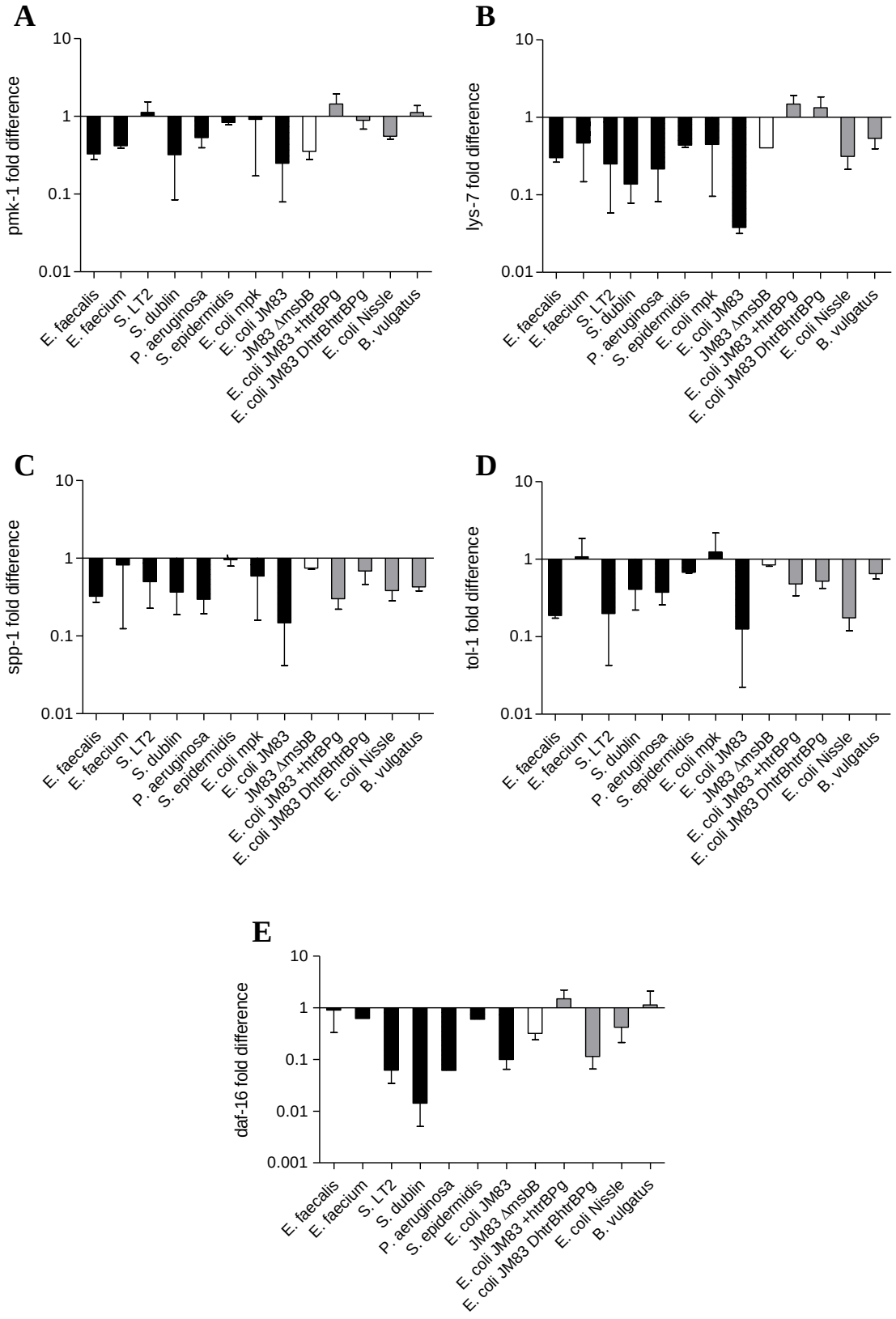

Figure 14. Relative mRNA expression of *C. elegans* after bacteria exposure. Worms were exposed to different bacteria species as shown on x axis for 24hours before RNA was extracted and measured by quantitative PCR using primers for genes of interest as indicated in the respective title (A-E). Bars represent SEM.

4 Discussion

It is very difficult to assess the contributions specific to microorganisms on inflammation processes due to their complex interactions on many aspects of host physiology. Although the worms were treated by a bleaching procedure to free them of germs we did not verify whether the worms nor the NGM medium were indeed germ-free after bleach treatment. Regarding the developmental assays that were carried out before transferring worms to the different bacterial conditions e.g. for lifespan or fluorescent microscopy, the density of eggs was maintained by pipetting 10 µl of eggs in water, which is subjected to pipetting variation and does not accurately yield same densities. Housing densities may affect developmental physiology by overcrowding effects and thereby effect ROS production, lifespan, and immune signaling. Finally, it should be mentioned, that this research like investigations on any other organism might lead to private mechanisms only found in *C. elegans* that cannot be applied to humans. Nevertheless, due to conserved immune signaling pathways, *C. elegans* seems to be an appropriate model organism for gaining insights into microbial interactions (Cabreiro, Au et al. 2013). Importantly some aspects of microbial contributions on life span and immune signaling have already been shown to be similar between worms and mammals: for example research has found beneficial effects of probiotics (i.e. bacteria in the diet that alter microbial composition in a beneficial manner) including immunomodulation in humans. Similarly for *C. elegans* it was shown that probiotic bacteria (i.e. Bifidobacterium) can increase lifespan by enhanced p38 MAPK immune signaling (Komura, Ikeda et al. 2013). Additionally there are functional and morphological similarities between the worm's intestine and the human gut (Irazoqui, Urbach et al. 2010). We are therefore optimistic that findings in *C. elegans* will contribute towards a better understanding of microbial contributions to inflammation processes in higher organisms as well.

In an effort to elucidate the contributions of bacteria during intestinal inflammation we assessed effects of different bacteria strains on overall lifespan, bacterial avoidance behavior, *sod-3* gene expression, ROS production, and on immune parameters. The rationale for this study was the search for an appropriate model system that could be used as a cost and time efficient high throughput screening to carry out explorative studies to tackle both sides of the host pathogen equation e.g. to identify bacterial and host factors in *C. elegans* before testing promising candidates in mouse models.

In our experiments we found that different bacterial species exert different effects on overall lifespan of *C. elegans*. Bacteria of the phyla *Salmonella* (*S.* LT2 and *S.* Dublin) have the most detrimental impact on lifespan of wild type *C. elegans* while the bacteria strain *E. coli* JM83 show protective effects by prolonging overall lifespan. *Salmonella* species have been described as known pathogens for *C. elegans* (Aballay, Yorgey et al. 2000, Labrousse, Chauvet et al. 2000), which is in accordance with our findings. Contrarily there is no data on *C. elegans* infection with *E.coli* JM83. However, findings in mouse models have shown that *E. coli* JM83 induces intestinal inflammation in mice (Gronbach, Flade et al. 2014), which contradicts our results or points to a private mechanism in *C. elegans*.

In the food choice assay bacteria of the phyla *Staphylococcus* and *Enterococcus* (*S. epidermidis*, *E. faecalis*, and *E. faecium*) evoke the highest avoidance behavior in *C. elegans*. As it was discussed that *C. elegans'* olfactory avoidance behavior can be used to avoid pathogens (Zhang, Lu et al. 2005) we presented known pathogens as well as bacteria to be tested for their pathogenic potential. *C. elegans* clearly avoids *E. faecalis*, which in the literature has been well described as pathogen of *C. elegans* (Garsin, Sifri et al. 2001). Moreover, in earlier experiments *E. faecalis* has been portrayed to provoke intestinal inflammation (Chavez, Mohri-Shiomi et al. 2007). Therefore this finding is particularly interesting as it suggests that worms do avoid bacteria that are responsible for intestinal inflammation. *E. faecium*, on the other hand, does not cause significant killing in survival assays with *C. elegans* (Garsin, Sifri et al. 2001). It should therefore not be considered as pathogen but was nevertheless avoided in our experiments. The species *E. coli* Nissle and *P. aeruginosa* are most appealing to *C. elegans* in the food choice assay. Although there is no data on *C. elegans* infection with *E. coli* Nissle, it is well-known as a probiotic strain ("Mutaflor"). Other probiotic bacteria were already shown to be beneficial to health in *C. elegans* (Komura, Ikeda et al. 2013). Our findings therefore support the observation that probiotics function alike in humans as they do in worms and that *E. coli* Nissle does not only exert its probiotic effect on humans but also on *C. elegans*. However, it is somewhat surprising that *P. aeruginosa* also attracts *C. elegans*. If *C. elegans* was capable of olfactory avoiding pathogens it should avoid *P. aeruginosa* as it has been well established to kill *C. elegans* (Tan, Mahajan-Miklos et al. 1999). Indeed, there might be a different process

in place, as *P. aeruginosa* is well known for its lime blossom scent. It would be interesting to further study the neuronal networks underlying this mechanism.

The ROS production analysis showed that worms exposed to *E. coli* JM83 or worms that were not exposed to bacteria at all compared to worms feeding on *E.coli* OP50 had significantly less ROS production. Interestingly, *C. elegans* exposed to *E. coli* OP50 had the highest ROS production out of all conditions. These results are consistent with the observations from the lifespan assays and point to a protective effect of *E. coli* JM83 and a detrimental effect of *E. coli* OP50 on overall fitness that might be mediated by ROS production. Unfortunately, there are no studies on *E. coli* JM83 carried out in *C. elegans* in the literature. Our results on *E. coli* OP50 however emphasize what has been mentioned in various publications: *E. coli* OP50 as the standard laboratory bacteria associated with *C. elegans* does have detrimental effects on fitness and lifespan in *C. elegans* (Garigan, Hsu et al. 2002, Garsin, Villanueva et al. 2003, Chavez, Mohri-Shiomi et al. 2007, Komura, Ikeda et al. 2013).

Our SOD-3 expression analysis by fluorescent microscopy revealed that worms fed on *S.* Dublin, *S.* LT2, and *E. faecalis* had higher SOD-3 expression than worms feed on *E.coli* OP50. This observation on *Salmonella* nicely fits to our data from the lifespan experiments. Although *Salmonella* are known bacterial pathogens of *C. elegans* there is no evidence in the literature whether this effect is mediated through intestinal inflammation. Here we propose that the detrimental effects of *Salmonella* on lifespan of *C. elegans* might be mediated through intestinal inflammation, which in turn leads to an upregulation of SOD-3. Unfortunately, we did not test ROS production in *C. elegans* after *Salmonella* exposition, which should be evaluated in future studies to confirm our hypothesis.

Finally, we showed that *C. elegans* exposure to all bacteria species resulted in equal or lower mRNA expression of different immune parameters compared to exposure to *E. coli* OP50. It is noticeable that exposure to *E. coli* JM83 resulted in significantly lower mRNA expression of all genes. This result on *E. coli* JM83 is consistent with the observations from the lifespan assay and ROS quantification experiment and might explain the underlying mechanism. We propose a private mechanism of *C. elegans* by which *E. coli* JM83 might promote longevity in *C. elegans* by protection against inflammation. The results on increased immune signaling in worms exposed to

E. coli OP50 are surprising but also in part engraft into our results on elevated ROS production after exposure to *E. coli* OP50.

This study was meant to evaluate *C. elegans* as a potential model organism to study bacteria-host interactions by characterizing the role of intestinal microorganism in the inflammation process. Elucidating the underlying cell signaling pathways by which bacteria might induce or perpetuate intestinal inflammation are beyond the scope of this work but would be a future area of application for this model. However, some of our results do not agree with what is published in the literature and therefore seem to be questionable. However, our results are conclusive in that they show that worms fed with *E. coli* JM83 live longer, show a lower ROS production, and lower immune signaling which points to an underlying mechanism connecting these findings. At the same time worms feeding on *S.* LT2 or *S.* Dublin had significantly shorter lifespan and higher SOD-3 induction also pointing to a conclusive mechanism. Unfortunately, most of our results show no clear difference between exposition to *E. coli* OP50 and other bacteria which might be due to low animal numbers. However, in summary our results seem conclusive but contrary to the literature. This inconsistency might be explained by our findings on *E. coli* OP50 resulting in increased ROS production and immune signaling. We used *E. coli* OP50 as baseline or standard in all experiments; but if the standard also shows upregulation of inflammation parameters all the values in relation to standard will be biased. This raises the question whether *E. coli* OP50 is an adequate base line parameter for experiments on inflammation in *C. elegans.*

Our study showed that *E. coli* OP50 might have detrimental effects on *C. elegans,* which is debated controversially in the literature. In all studies on *C. elegans* use *E. coli* OP50 was used as standard to compare other bacteria to. Very few studies share the observation that *E. coli* OP50 is slightly pathogenic to *C. elegans* (Garsin, Sifri et al. 2001)). “The dosage makes the poison” (Paracelsus) might describe the situation best: high bacterial numbers might have different effects on *C. elegans’* health and inflammation parameters regardless of their inflammatory potential. Many working techniques for *C. elegans* such as preparation of NGM plates are well established in the literature. However during the literature research for this work not one paper was found that contained specification of bacterial numbers or densities. At worst, these are reported simply as 'standard' preparation of NGM plates. More often, the recipe from

www.wormbook.org is given but without the method of preparation of the actual bacterial culture or source of its components. Such culture variations could explain inconsistent experimental outcomes between laboratories.

We strongly believe that bacterial numbers can have an influence on the pro- or anti-inflammatory potential they exert on *C. elegans*. We therefore carried out our study with identical bacterial numbers as measured by optical density. We arbitrarily chose an optical density (OD_{600}) of 2 representing bacterial number of $1x10^9$. However, this value was chosen at random and not assessed in a study. Also no further characteristics were collected about bacterial fitness. Bacterial fitness depends on various factors e.g. fitness for *E. coli* might vary depending on which medium it grows. Indeed, it was observed that worms grown on *E. coli* OP50 in BHI medium live significantly shorter than when cultivated on *E.coli* OP50 on NGM plates (Garsin, Sifri et al. 2001). We therefore propose a bacterial fitness index which takes factors such as temperature, O_2 content, nutrients, and pH among others into account to calculate bacterial numbers that will meet the same "fitness level". We strongly believe that the pathogenic potential of bacteria depends on their fitness, e.g. pathogenic bacteria that are grown under suboptimal conditions might even be harmless to *C. elegans*. Alternatively, as mentioned earlier, we did not assess whether *C. elegans* was indeed germ-free. If *C. elegans* was "contaminated" with other bacteria species than the one of choice this flora might have superseded pathogenic bacteria due to lower fitness or vice versa. Further tests to check for contamination e.g. by plating *C. elegans* on LB medium or adding specific antibiotics to the NGM medium might be helpful to control for contamination. Furthermore what happens to *E. coli* OP50 or for that matter with any bacteria when consumed by *C. elegans*, particularly digestion in the intestinal lumen, remains poorly understood (Cabreiro, Au et al. 2013). For example it would be interesting to evaluate whether bacteria are still alive once they are ingested by *C. elegans*.

It therefore seems indispensable to first learn more about culture conditions and bacterial fitness inside and outside of *C. elegans* before investigating bacterial effects on *C. elegans'* fitness.

In summary, the present study suggests that fluorescent-based screenings of *C. elegans* are a potential high throughput method to benefit the elucidation of bacterial virulence factors that are responsible for intestinal inflammation. This method appeals as a cost and

time efficient tool and does not seem too elaborate for a routine lab. However, it seems that it is too early to ask the right question for a high throughput screening before carefully understanding the basics. Once the basic mechanisms that are discussed above are understood fluorescent based screenings appeal as an elegant tool into host microbial interactions particularly in *C. elegans* that might help to promote the exact contribution of bacteria to intestinal inflammation processes and permit diagnostic and therapeutic advances in the area IBD research.

5 Summary

Microorganisms play an important role in various physiological reactions and perturbations may contribute to the etiology of diseases like Inflammatory Bowel Disease (IBD). *Caenorhabditis elegans* is a promising model for studying the distributions of certain bacteria in IBD. However, knowledge on the effects of bacteria on its immune system is still rudimentary e.g. it is currently investigated whether pathogens contribute to inflammation because they have a metabolic advantage during colonizing of the gut.

In an effort to elucidate the contributions of bacteria during intestinal inflammation we assessed effects of different bacteria strains on overall lifespan, bacterial avoidance behavior, *sod-3* gene expression, ROS production, and immune parameters.

Salmonella are known bacterial pathogens of *C. elegans* but there is no evidence in the literature whether this effect is mediated through intestinal inflammation like in humans. We found that worms exposed to *Salmonella* species had shorter life span. We suggest that this is mediated through intestinal inflammation, which in turn leads to an upregulation of SOD-3 as confirmed by fluorescent microscopy. Furthermore, for the first time, colonization of C. *elegans* with *E. coli* JM83 was investigated: we observed that *E. coli* JM83 colonization promotes longevity, e.g. a longer lifespan, in C. *elegans.* Our results also propose that longevity might be mediated by protecting against inflammation, as analyzed by qPCR of prominent immune signaling pathways, and by downregulation of ROS production. However, this is in contrast to what is known from mouse models and therefore points to a private mechanism of *C. elegans*.

Surprisingly we also found that immune parameters and ROS production was elevated after exposure to *E. coli OP50*, bacteria used as standard food source for *C. elegans* in the lab. A critical requirement for research into bacterial contributions is a well-defined and consistent system. There is currently no complete understanding of what effects *E. coli OP50* (and other bacteria) have on *C. elegans'* physiology and whether these effects vary depending on numbers and fitness of the respective bacteria. Our research has proven *C. elegans* as a promising model in the context of IBD because human enteric pathogens, e.g. Salmonella, lead to intestinal inflammation. However, it seems indispensable to first learn more about culture conditions and bacterial fitness inside and outside of *C. elegans* before investigating bacterial effects on host physiology.

Zusammenfassung

Das Mikrobiom spielt in vielen Stoffwechselwegen eine zentrale Rolle. Ein Ungleichgewicht kann in genetisch prädisponierten Individuen zu Krankheiten wie zum Beispiel chronisch-entzündlichen Darmerkrankungen (CED) führen.

Caenorhabditis elegans ist ein vielerforschtes Tiermodel, welches auch zur Erforschung der Rolle von Darmbakterien in der Pathogenese von CED von großer Nützlichkeit sein könnte. Bislang ist die Bedeutung der Darmbakterien für das Immunsystem von *C. elegans* jedoch nicht vollständig geklärt. Es ist denkbar, dass Pathogene eine Entzündung begünstigen, in dem sie durch metabolische Vorteile den Darm effizienter besiedeln können.

Um die Auswirkungen des Mikrobioms auf Darmentzündungen zu erforschen, wurden in der vorliegenden Arbeit der Effekt der Bakterien auf Lebensspanne, im food choice assay, *sod-3* Genexpression, Produktion von reaktiven Sauerstoffspezies (ROS), und Immunparametern untersucht.

Salmonellen sind auch für *C. elegans* Krankheitserreger, aber es ist bislang nichts über die zu Grunde liegende Pathogenese bekannt. Wir konnten zeigen, dass eine Salmonella-Exposition eine kürzere Lebensspanne bei *C. elegans* bedingt und, dass diese durch Entzündungsprozesse im Darm ausgelöst wird, was durch eine erhöhte SOD-3 Produktion gezeigt werden konnte. Darüber hinaus haben wir erstmalig eine Kolonisation von *E. coli* JM83 in *C. elegans* untersucht: es wurde beobachtet, dass *E. coli* JM83 zu einer verlängerten Lebensspanne führt, vermutlich durch Schutz vor Entzündung. Dies konnte an Hand einer qPCR Analyse von charakteristischen Immunparametern, die nach einer *E. coli* JM83 Exposition herunter reguliert waren, gezeigt werden. Diese Ergebnisse widersprechen Erkenntnissen aus dem Maus-Modell und weisen daher auf einen eigenen Signalweg in *C. elegans* hin.

Interessanterweise fanden wir auch, dass sowohl diese Immunparameter, als auch die ROS-Produktion in *C. elegans* erhöht war, nachdem die Nematoden *E. coli OP50* ausgesetzt waren. Bei *E. coli OP50* handelt es sich um einen Bakterienstamm der standardmäßig als Nahrungsquelle für *C. elegans* im Labor eingesetzt wird. Eine entscheidende Voraussetzung für die Erforschung von Bakterieninteraktionen ist ein stabiles und eindeutiges System. Derzeit sind sowohl die Auswirkungen von *E. coli OP50* (und andere Bakterien) auf die Physiologie von *C. elegans*, als auch die

Frage, ob die bakteriellen Auswirkungen dosis- oder fitnessabhängig sind, nicht verstanden.

Unsere Arbeit hat gezeigt, dass *C. elegans* ein bedeutendes Model für die Erforschung von CED sein kann, weil beispielsweise der menschliche Pathogen Salmonella auch in *C. elegans* zu entzündlichen Prozessen im Darm führt. Dennoch scheint der primäre Schritt zu sein, die Kulturbedingungen und Fitness von Bakterien innerhalb und außerhalb von *C. elegans* zu verstehen, bevor eine Untersuchung der bakteriellen Bedeutung im Entzündungsgeschehen erforscht werden kann.

6 Literature

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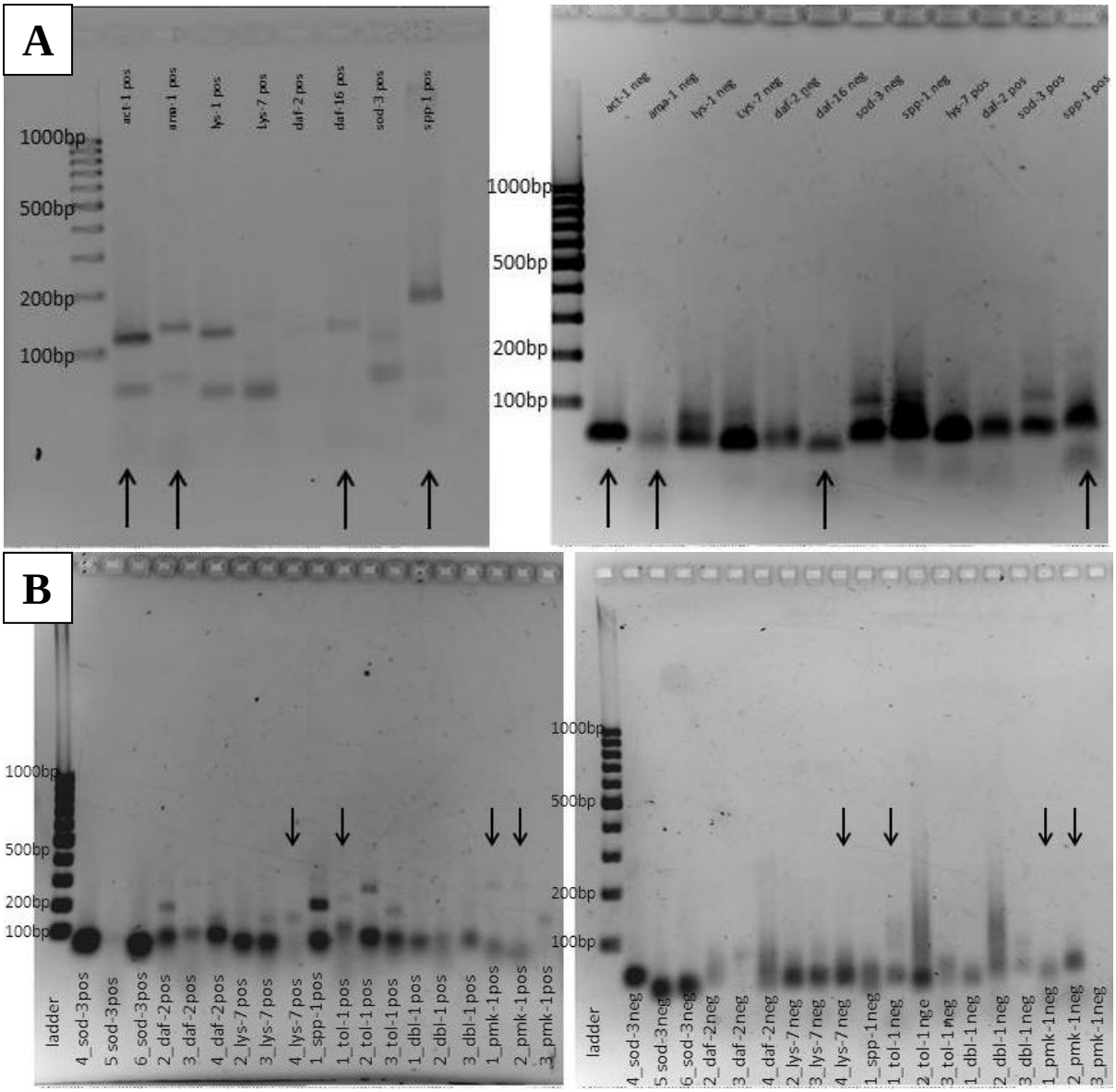
7 Supplementary Information

Supplementary Table 1. Primer pairs used for gel electrophoresis. These primer pairs were tested in PCR and gel electrophoresis whether they yielded the expected gene length. Numbers in front of the gene name indicate that multiple primer pairs were tested. "F" or "r" at the end of the gene name indicate forward and reverse primer. The Table shows the primer sequences, where the sequence was obtained from, the amplicon size and whether the desired amplicon size was seen in the gel electrophoresis. Primers for which the desired length was obtained are in bold type.

Gene	primer sequence	source	Amplicon Size	Desired amplicon size?
act-1_f	**GCTGGACGTGATCTTACTGATTACC**	**(Hoogewijs, Houthoofd et al. 2008)**	**114**	**yes**
act-1_r	**GTAGCAGAGCTTCTCCTTGATGTC**			
ama-1_f	**CCTACGATCTATCGAGGCAAA**	**(Hoogewijs, Houthoofd et al. 2008)**	**129**	**yes**
ama-1_r	**CCTCCCTCCGGTGTAATAATG**			
lys-1_f	GGATTCAGGTTACCTCCCCAGCC	(Alper, McBride et al. 2007)	116	no
lys-1_r	GGTGTAGATTCCGACAGTCAGTCCG			
lys-7_f	GTCTCCAGAGCCAGACAATCCGG	(Alper, McBride et al. 2007)	143	no
lys-7_r	CCAGTGACTCCACCGCTGTACAC			
daf-2_f	GGCCGATGGACGTTATTTTG	(Zhang, Jie et al. 2009)	115	no
daf-2_r	TTCCACAGTGAAGAAGCCTGG			
daf-16_f	**TTTCCGTCCCCGAACTCAA**	**Zhang, Jie et al. 2009**	**116**	**yes**
daf-16_r	**ATTCGCCAACCCATGATGG**			
sod-3_f	AGCATCATGCCACCTACGTGA	Zhang, Jie et al. 2009	171	no
sod-3_r	CACCACCATTGAATTTCAGCG			
spp-1_f	GATGATCTCGATGCATGGCTTGATG	(Anyanful, Easley et al. 2009)	157	no
spp-1_r	CCTTGCACGCCTTGTCTGGAGAATCC			
1_sod-3_f	ACATGCATGCAGTTTTTCATTTTCG GTATCGAAAACC	(Doonan, McElwee et al. 2008)		no
1_sod-3_r	GGGGTACCGGTTGTCGAGCATTG GCAAATCTCTCG			
2_sod-3_f	AATGCTGCAATCTACTGCTC	(Honda and Honda 1999)		no
2_sod-3_r	AGCGTTTTAAACTACATCTG			
3_sod-3_f	CCAACCAGCGCTGAAATTCAATGG	(DePina, Iser et al. 2011)		no
3_sod-3_r	GGAACCGAAGTCGCGCTTAATAGT			
1_daf-2_f	GGCACCGGTGCGGGAGCATTGAAA CGAACAAAACACATC	(Dillin, Crawford et al. 2002)		no
1_daf-2_r	TCCAGCACATTTTCATCACCTTATACC			
1_daf-2_f	AATTCCCATAAATTTGTGTGTGG	http://gfpweb.aecom.yu.edu/qgene		no
1_daf-2_r	ATTGATTCTCGTCATCGTTCTGT			
gfp_f	GATATCATGAGTAAAGGAGAAGA ACTTTTCA	(Li and Wang 2012)		no
gfp_r	TCTAGATTACTTGTATGGCCGGC TAGCGAAT			
1_lys-7_f	GTCTCCAGAGCCAGACAATCC	(Marsh, van den Berg et al. 2011)		no
1_lys-7_r	CCAGTGACTCCACCGCTGTA			
4_sod-3_f	CTCCAAGCACACTCTCCCAG	NCBI Primer Blast	307	no
4_sod-3_r	ACCGAAGTCGCGCTTAATAGT			
5_sod-3_f	TGGACACTATTAAGCGCGAC	NCBI Primer Blast	154	no
5_sod-3_r	GGATCCTGGTTTGCACAGGT			
6_sod-3_f	GCAATTGCTCTCCAACCAGC	NCBI Primer Blast	134	no
6_sod-3_r	CCGAAGTCGCGCTTAATAGTG			
2_daf-2_f	TGGGTCACGAAGATCAGCAG	NCBI Primer Blast	183	yes

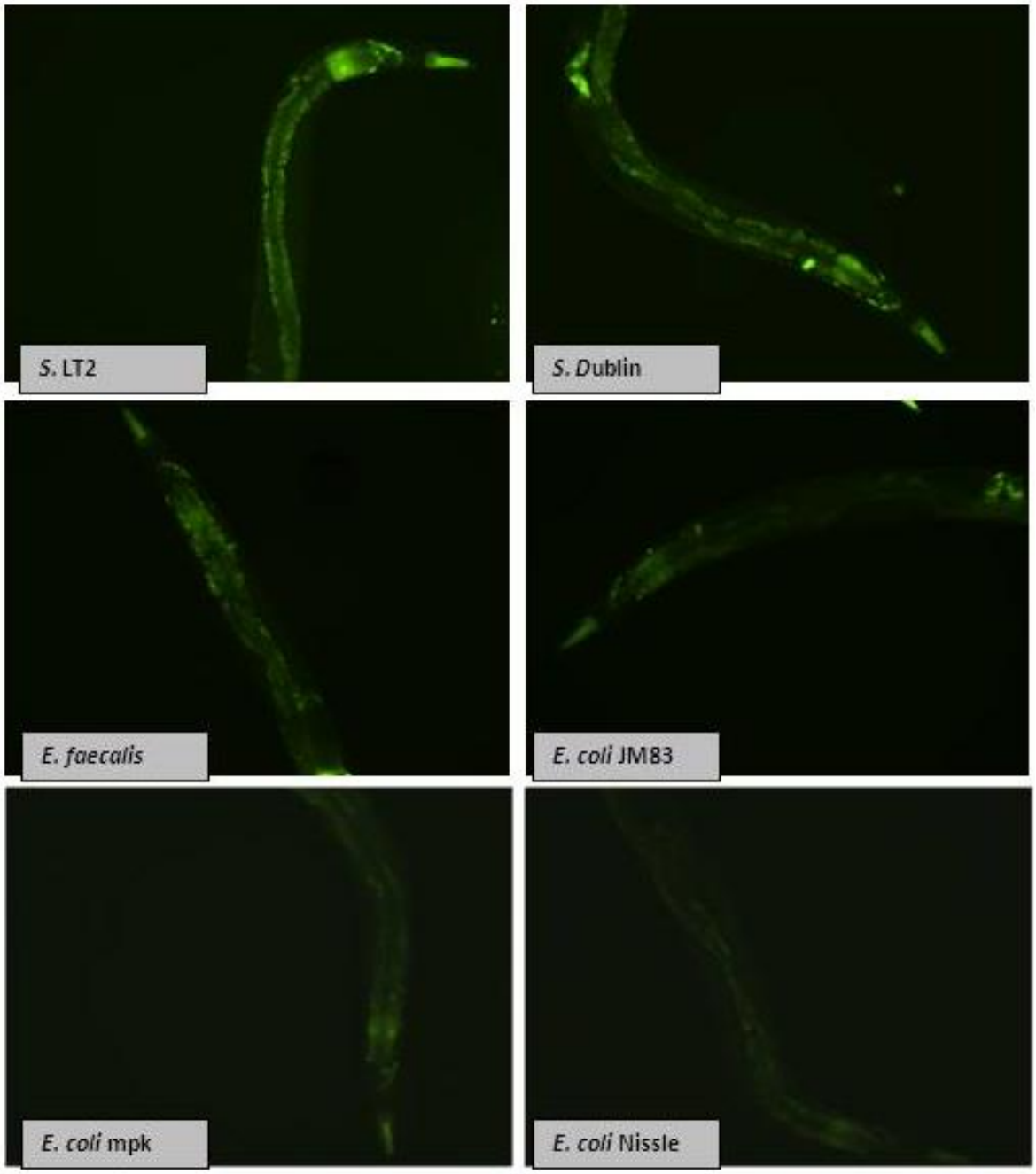
2_daf-2_r	CTCGCATCGTGTCTCTCGG			
3_daf-2_f	ATGCCGAGAGACACGATGC	NCBI Primer Blast	277	no
3_daf-2_r	AGCTTCCGGGTAAAGGTTCG			
4_daf-2_f	TAATGCCGAGAGACACGATGC	NCBI Primer Blast	121	no
4_daf-2_r	GGCTTCTTTCCACCGAGAGT			
2_lys-7_f	TCGGCATCAGTCAAGGTTCC	NCBI Primer Blast	207	no
2_lys-7_r	TGGGTTGTATGCACGAACGA			
3_lys-7_f	**GTGCAGTTTTCGTTCGTGCAT**	**NCBI Primer Blast**	**138**	**yes**
3_lys-7_r	**GCTTGTTTGAAACGGGCTGT**			
4_lys-7_f	**TGTCTCCAGAGCCAGACAATC**	**NCBI Primer Blast**	**136**	**yes**
4_lys-7_r	**TCCACCGCTGTACACATTCC**			
1_spp-1_f	**CTCTCGTCGAGGGTGGAGA**	**NCBI Primer Blast**	**197**	**yes**
1_spp-1_r	**ATCAACATCCTTGCACGCCT**			
1_tol-1_f	**GGATAGTGGGATGCGAGACC**	**NCBI Primer Blast**	**220**	**yes**
1_tol-1_r	**TGTAAATGCGGCAGAGAGCA**			
2_tol-1_f	**CTGCTAACGGTGACGAATGC**	**NCBI Primer Blast**	**253**	**yes**
2_tol-1_r	**GTCTCGCATCCCACTATCCG**			
3_tol-1_f	**TAAGCTGACAGAAGTGCCCG**	**NCBI Primer Blast**	**167**	**yes**
3_tol-1_r	**TGTAGGGGCCGATACTTTGC**			
1_dbl-1_f	CATCATAACACCGAGGCCGA	NCBI Primer Blast	228	no
1_dbl-1_r	GCAAGGTGGCGGTACTTCAT			
2_dbl-1_f	TGCTTCGGAAGTTGGGACTC	NCBI Primer Blast	243	no
2_dbl-1_r	TGCGTCGTAACCGGAGTTTC			
3_dbl-1_f	TTATGGCACCCAAGGGCTAC	NCBI Primer Blast	157	no
3_dbl-1_r	TCAGTAGGCACACAGCAAGG			
1_pmk-1_f	**TCGCCGTGATTTCAAACGTC**	**NCBI Primer Blast**	**267**	**yes**
1_pmk-1_r	**GCAAATGCCACGTTTTTCTGGA**			
2_pmk-1_f	**GGAACTGTTTGTGCTGCTGAA**	**NCBI Primer Blast**	**272**	**yes**
2_pmk-1_r	**TGGTCATCGTTGAGTCGCTG**			
3_pmk-1_f	CTTCATCCGACTCCACGAGA	NCBI Primer Blast	129	no
3_pmk-1_r	TTCAGCAGCACAAACAGTTCC			
1_gfp_f	GTCAGTGGAGAGGGTGAAGG	NCBI Primer Blast	244	no
1_gfp_r	CATAACCTTCGGGCATGGCA			
2_gfp_f	GAGAGGGTGAAGGTGATGCAA	NCBI Primer Blast	236	no
2_gfp_r	ATAACCTTCGGGCATGGCAC			
3_gfp_f	GCCATGCCCGAAGGTTATGT	NCBI Primer Blast	462	no
3_gfp_r	GGCAGATTGTGTGGACAGGTA			



Supplementary Figure 1. PCR analysis on whole worms using primer pairs as indicated. Worms were sampled after exposition to different bacteria for 24 hours; RNA was pooled from different conditions. Positive (pos; with RNA) controls are on the left, negative controls (neg; without RNA) are shown on the right. Marker ladder is to the left of each image and bands appeared as seen on the gel. Primer used for further experiments are indicated by black arrows. Primers with the expected amplicon length from left to right are in image A: act-1 (114bp), ama-1 (129bp), daf-16 (116) and spp-1 (157bp). In image B: lys-7 (136bp), 1-tol-1 (220bp), 1-pmk-1 (267bp) and 2-pmk-1 (272bp). 1-pmk-1 and 2-pmk-1 are two primer pairs for the same gene as indicated by the number in front of the gene name.

Supplementary Table 2. Light cycler analysis of primer pairs. After primer pairs were shown to amplify the desired gene length they were further analyzed for amplification specificity. Efficiencies and errors as well as Cp values for the different samples are shown. The Table also shows the dilution in which the primer was used in further experiments.

Gene	Efficiency and error from Standard curve	Cp values	Used dilution of primer
Ama-1	2,463 +/- 0,0240	Standard undiluted 19,00 Standard 1:5 diluted 20,47 Standard 1:25 diluted 22,54 Standard 1:125 diluted 24,82 Control sample 35,00 Negative sample 18,68	1:10 dilution
Act-1	2,062 +/-0,0152	Standard undiluted 12,47 Standard 1:5 diluted 14,34 Standard 1:25 diluted 16,51 Standard 1:125 diluted 18,79 Control sample 33,22 Negative sample 12,46	1:10 dilution
1-pmk-1	2,165 +/- 0,0231	Standard undiluted 20,02 Standard 1:5 diluted 21,75 Standard 1:25 diluted 25,15 Standard 1:125 diluted 26,26 Control sample 28,94 Negative sample 20,13	undiluted or 1:10 dilution
1-tol-1	2,507+/-0,0122	Standard undiluted 19,21 Standard 1:5 diluted 20,81 Standard 1:25 diluted 22,70 Standard 1:125 diluted 24,14 Control sample 25,20 Negative sample 19,59	undiluted or 1:10 dilution
4-lys-7	2,510+/- 0,0177	Standard undiluted 19,58 Standard 1:5 diluted 21,11 Standard 1:25 diluted 23,06 Standard 1:125 diluted 25,46 Control sample 19,27 Negative sample 20,86	undiluted or 1:10 dilution
spp-1	2,764+/-0,00316	Standard undiluted 17,29 Standard 1:5 diluted 18,84 Standard 1:25 diluted 20,46 Standard 1:125 diluted 22,02 Control sample 17,21 Negative sample 23,48	undiluted
Daf-16	2,207 +/- 0,0419	Standard undiluted 18,63 Standard 1:5 diluted 21,19 Standard 1:25 diluted 22,58 Standard 1:125 diluted 24,59 Control sample 31,50 Negative sample 19,18	1:10 dilution



Supplementary Figure 2. Fluorescence microscopy of reporter *C. elegans.* Fluorescent microscopy was performed after exposure to different bacteria strains. SOD-3::gfp worms in the L4 stadium were exposed to *E. coli* OP50, *S.* LT2, *S.* Dublin, *E. faecalis*, *E.coli* JM83, *E.coli* mpk, and *E.coli* Nissle for 24 hours prior to microscopy. Images were taken to compare fluorescent levels after exposure to different bacterial strains. Fluorescent images were taken with an epi-fluorescent microscope, magnification 10x.

Erklärung zum Eigenanteil der Dissertationsschrift

Die Arbeit wurde im Institut für Medizinische Mikrobiologie und Hygiene unter Betreuung von Prof. Dr. med. Julia-Stefanie Frick durchgeführt.
Die Konzeption der Studie sowie der Experimente erfolgte von mir in Zusammenarbeit mit Frau Prof. Julia Frick und Anna Lange.
Die Einarbeitung in Laborabläufe und allgemeine Verfahren (z.B. PCR, Fluoreszenzmikroskopie) erfolgte vor allem durch Anna Lange, Andrea Schäfer und Annika Benders. Ich habe mich eigenständig und alleine um die Kultivierung von *C. elegans* und allen für die Experimente benötigten Bakterien gekümmert. Alle Experimente (Lifespan assay, Food choice Experiment, ROS Analyse, qPCR, Fluoreszenz Mikroskopie) und Experimentdurchläufe wurden von mir eigenständig konzipiert und durchgeführt. Alle für die Experimente benötigten Materialien (z.B. Primer Design) wurden von mir eigenständig bereitgestellt. Die statistische Auswertung erfolgte ebenfalls eigenständig durch mich.
Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Tübingen, den

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