From pathogen to a commensal: modification of the *Microbacterium nematophilum-C. elegans* interaction during chronic infection by the absence of host insulin signalling.

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ABSTRACT The nematode worm *Caenorhabditis elegans* depends on microbes in decaying vegetation as its food source. To survive in an environment rich in opportunistic pathogens, *C. elegans* has evolved an epithelial defence system where surface-exposed tissues such as epidermis, pharynx, intestine, vulva and hindgut have the capacity of eliciting appropriate immune defences to acute gut infection. However, it is unclear how the worm responds to chronic intestinal infections. To this end, we have surveyed *C. elegans* mutants that are involved in inflammation, immunity and longevity to find their phenotypes during chronic infection. Worms that grew in a monoculture of the natural pathogen *Microbacterium nematophilum* (CBX102 strain) had a reduced lifespan and vigour. This was independent of intestinal colonisation as both CBX102 and the derived avirulent strain UV336 were early persistent colonisers. In contrast, the long-lived *daf-2* mutant was resistant to chronic infection, showing reduced colonisation and higher vigour. In fact, UV336 interaction with *daf-2*, resulted in a host lifespan extension beyond OP50, the *E. coli* strain used for laboratory *C. elegans* culture. Longevity and vigour of *daf-2* mutants growing on CBX102 was dependent on the FOXO orthologue DAF-16. Our results indicate that the interaction between host genotype and strain-specific bacteria determines longevity and health for *C. elegans*.
INTRODUCTION

Bacteria associated with the animal gut are important for gastrointestinal function (Fischbach, 2018). Intestinal bacteria contribute to metabolic activities and are involved in the absorption of nutrients, protection of mucosal surfaces and the regulation of the immune function of the gut (Fischbach, 2018). Quantitative and/or qualitative alteration of the intestinal microbiota underline many inflammatory diseases as well as chronic gastrointestinal infections (CGIs), the latter being amongst the most common chronic diseases worldwide (Drossman et al, 2016). In the short term, CGIs can lead to altered mucosal and immune function (Drossman et al, 2016). In the longer term, CGIs cause impaired epithelial barrier function (a major factor of reduced health span in old age) and changes in intestinal microbiota (dysbiosis) that can “drive” constitutive inflammation in conditions like intestinal bowel disease and enterocolitis (Caravan et al, 2014). Moreover, sustained inflammation can lead to intestinal cancer or may accelerate age-dependent neurodegeneration (Caravan et al, 2014). In this context, understanding how host genetics interacts with the intestinal microbiota in health and disease is an important aspect in managing long-term health span. However, it is also a complex problem with many biological parameters.

Accumulating evidence indicates that the health-disease balance in CGIs is determined by the interaction of four components (Stecher et al, 2015). These are 1) the infectious agent inducing the disease, 2) host genetics that will influence mucosal barrier function and pro or anti-inflammatory responses, 3) the intestinal microbiota that can drive the disease when its composition change and 4) diet, which influences all other components. Negative interaction of these factors can abolish normal intestinal barrier function leading to constant mucosal inflammation and reduced
health and life expectancy (Finch, 2010). In contrast, non-inflammatory management can lead to extension of lifespan and healthspan (Hooper and Gordon 2001). It is evident that interactions of the above 4 components generate a complex set of conditions, which makes it hard to untangle the layers of chronic disease and arrive at causality.

In the simplified system that the nematode worm, Caenorhabditis elegans (C. elegans), is used in research laboratories around the world, the animal develops, feeds and ages in a bacterial monoculture. This means that food=microbiota=-pathogen (or commensal) depending on the choice of bacterium. This condition ensures the ability to modify host genetics in vivo by keeping all other parameters important for CGIs in control. When the pathogen changes, so will the function of diet and microbiota, and thus the system enables in principle to find the host genes that interact with a specific bacterial strain. In the wild, C. elegans is a bacterial feeder spending much of its life in decomposing vegetable matter and depends on microbes for food (Frezal and Felix 2015). These microbes are ground by the pharynx before they subsequently enter the gut. To survive in an environment rich in potentially damaging microorganisms, C. elegans has evolved an epithelial defence system coupled with the ability to discriminate between pathogenic vs. edible bacteria (reviewed in Kim and Ewbank, 2018).

Important antimicrobial molecules participating in these defences include a group of proteins called invertebrate lysozymes (ILYS) and in particular ILYS-3, which is expressed in both the pharynx and the intestine (O’Rourke et al, 2006). ILYS-3 (invertebrate-specific but related to human epithelial antimicrobial peptides) contributes to the digestion of the large amount of peptidoglycan fragments generated by the worm’s bacterial diet (either pathogenic or non-pathogenic).
(Gravato-Nobre et al, 2016). Loss of ilys-3 results in colonization of undigested bacteria from day 1 of adulthood in contrast to wild type worms (Gravato-Nobre et al, 2016). The latter only display colonization at very late stages of their life (Gravato-Nobre et al, 2016). Increased bacterial colonization in ilys-3 mutants leads to a significant lifespan reduction (Gravato-Nobre et al, 2016).

The isolation of natural bacterial pathogens of C. elegans has permitted a glimpse of the defence mechanisms employed by the worm as well as the host-pathogen interactions triggering such mechanisms (see Hodgkin et al, 2000; Nicholas and Hodgkin 2004; Hodgkin et al 2013). One such pathogen is Microbacterium nematophilum (Hodgkin et al, 2000). This Gram-positive bacterium adheres to the rectal and anal cuticle (Hodgkin et al, 2000) and induces inflammation, anal-region infection and tail swelling (Hodgkin et al, 2000; Parsons and Cipollo, 2014). Despite the fact that the most obvious response to infection is rectal colonization and the induction of inflammation in the rectal tissues, this bacterium also establishes itself in the gut of the worm. In fact, host lethality caused by M. nematophilum is due to gut infection rather than rectal inflammation (Parsons and Cipollo 2014). This makes it a good system to investigate effects that occur in the digestive tract associated with long-term gut colonization. In particular, to identify how long-term survival and health of the organism are influenced in the face of chronic intestinal infection.

To explore this question, we tested C. elegans mutants induced by chemical mutagenesis or targeted deletion in signalling pathways known to be involved in immunity to M. nematophilum infection and/or C. elegans longevity. These mutant worms were grown using solely the M. nematophilum strain CBX102 (where CBX102 is the sole source of food=microbiota=pathogen). Using CBX102, we were able to
separate estimated host survival probabilities into four categories in relation to ily-3 and wild type (N2) worms. We identified daf-2 as long-lived in conditions of chronic infection. Bacterial colonisation of CBX102 in N2 worms was increased compared to the laboratory E. coli strain OP50. However, colonisation in N2 per se was not the reason for pathogenesis as the non-virulent M. nematophilum strain UV336 did not curtail lifespan despite being able to colonise at the same levels as CBX102. Nevertheless, daf-2 worms were healthier and had reduced colonisation compared to normal worms. daf-2 health and longevity on CBX102 involved the canonical insulin signalling pathway and were thus dependent on the FOXO orthologue daf-16, like many other daf-2-mediated effects. Finally, the non-pathogenic UV336 was able to support an extended lifespan for daf-2 beyond that observed when using OP50. These results indicate the complex and strain-specific interactions between intestinal bacteria and host genetics.

RESULTS

Chronic Gastrointestinal Infection (CGI) curtails lifespan, reduces health and accelerates ageing in N2 worms. In our experimental CGI set-up, C. elegans develops, feeds and ages only with M. nematophilum, thus having the same microbial challenge from birth. Compared to standard laboratory food (E. coli strain OP50), the pathogenic M. nematophilum strain CBX102 reduced host lifespan (Fig. 1A) and health, measured by vigour of movement in liquid assays (Fig. 1B). The avirulent M. nematophilum UV336 strain (derived from CBX102 by UV mutagenesis, Akimkina et al, 2006), had the same level of bacterial colonisation as CBX102 (Fig. 1C) but in contrast to the latter, presented no negative impact on median lifespan (Fig. 1A) or health span (Fig. 1B) both of which were largely
comparable to OP50. In this context, two strains of the same species behaved one as a pathogen (CBX102) and one as a commensal (UV336). Moreover, CBX102 accelerated mitochondrial fragmentation (Fig S1), a sign of age-dependent stress in worms (Han et al, 2017).

**CGI defines four lifespan groups of *C. elegans* mutants**

To find worms that could outlive N2 under CGI while retaining their health, we tested *C. elegans* mutants induced by chemical mutagenesis, in signalling pathways known to be involved in immunity to infection and/or longevity. Our tests pertained to studying intestinal colonization, lifespan and health/vigour. All strains were cultured from eggs in pure CBX102 and tested for bacterial colonization.

The mutants tested were in genes of the p38 MAPK pathway (*sek-1, nsy-1, pmk-1, kgb-1*); TGF-β (*dbl-1*); ERK (*sur-2*), cuticle properties (*sqt-3*), bacterial killing (the lysozyme-encoding *lys-3* and *lys-7*), pharyngeal-defective with enhanced bacterial colonisation of the intestine (*phm-2*), stress-specific regulators (*hsf-1*), apoptosis (the *p53* homologue *cep-1* and *ced-1*) and lifespan determinants (*hif-1, vhl-1, age-1, eat-2, cik-1, daf-2*). In terms of host survival probabilities, CGI separated the mutants tested into four categories: A) Those whose lifespan was shorter than *ilys-3* mutants (Fig 2A); B) those that had lifespan comparable to *ilys-3* (Fig 2B); C) those with life expectancy comparable to N2 (Fig 2C); and D) those that had an increased lifespan compared to N2 (Fig 2D). Most of the time (but not always) bacterial colonisation negatively correlated with lifespan (Fig S2). Table S1 has a summary of alleles used categorised in the four groups as above (A-D) and includes results on lifespan, health (vigorous movement) and bacterial colonisation along with extracted p-values.
**daf-2 mutant is long-lived and healthier than N2 under CGI.**

From the mutants tested, only one mutant, in the insulin receptor, daf-2 was found to be living longer than N2 under CGI (Fig. 2D). This confirmed and extended observations for daf-2 longevity in OP50 (Kenyon *et al*, 1993) as well as acute infections by *S. aureus, P. aeruginosa* or *E. faecalis* (Garsin *et al*, 2003) and *Salmonella typhimurium* (Portal-Celhay *et al*, 2012). Bacterial colonisation of daf-2 was reduced compared to N2 (Fig. S2). It was also reduced compared to other normally long-lived mutants such as *age-1* (Fig S2). The latter is long-lived on OP50 (Friedman and Johnson, 1988) but had lifespan indistinguishable to N2 on CBX102. (Fig 2D). Finally, as Fig S3 shows, daf-2 worms did not display inflammatory tail swelling like that reported for N2 (Hodgkin *et al*, 2000).

Despite the adverse effects of CBX102 on N2 lifespan (when compared to OP50), N2 median lifespan on UV336 vs. OP50 was statistically indistinguishable (Fig. 3A). The survival pattern of daf-2 mutants on CBX102 was statistically comparable to that of daf-2 on *E. coli* OP50 (Fig. 3B). Compared to N2 on CBX102, daf-2 worms were still longer-lived (compare Fig. 3A and 3B). Notably, daf-2 lifespan was extended on UV336 compared to daf-2 on CBX102 even beyond the TD_{50} and maximum lifespan limits defined by OP50 (Fig 3B). This boosting effect on lifespan by UV336 over and above OP50 was not observed in N2 (Fig. 3A). This result showed that the genotype of the host can modify the effect of a bacterial strain and this interaction determines lifespan. Conversely, any effect of a bacterial species is strain specific.
**Daf-16 is required for the longevity and health of daf-2 mutants under CGI**

Lifespan extension through the DAF-2 insulin-signalling pathway in *C. elegans* occurs by de-repression of the fork-head transcription factor DAF-16, which is normally under negative regulation by DAF-2. Strong loss-of-function alleles of *daf-16* such as *mgDf47* and *mu86* suppressed the long-lived phenotype of *daf-2* under CGI with CBX102 making the double *daf-16; daf-2* statistically indistinguishable from N2 (Fig 4). Moreover, loss of DAF-16 suppressed the vigorous thrashing ability of *daf-2* making again the double *daf-16; daf-2* statistically indistinguishable in its vigour compared to N2 (Fig S4). As expected from the above, *daf-16* on its own, exhibited a comparable degree of survival to CGI as N2 worms. Therefore, in *C. elegans*, the DAF-2/DAF-16 axis is important for maintaining longevity and health under CGI by a natural pathogen.

**DISCUSSION**

We wanted to develop a simple model to test host longevity and health under CGI. *C. elegans* is such a model since microbiota=pathogen=food as the worm is a bacterial feeder and its laboratory culture is typically a bacterial mono-association.

Our work shows where longevity and immunity converge under CGI. Our data indicate that the insulin signalling pathway modulates intestinal colonisation to affect long-term host survival. How long the host will live however, is also dependent on the strain-specific pathogenicity of the bacteria on which *C. elegans* is feeding. The natural pathogen *M. nematophilum* strain CBX102 curtailed lifespan and health of N2 wild type worms but strain UV336 was statistically indistinguishable from *E. coli* OP50, the “normal” lab food. Inactivation of the insulin receptor in *daf-2*, made worms live longer and be healthier and physiologically
younger on CBX102. This correlated with reduced colonisation (Fig S3; Fig S5). In addition, UV336 extended daf-2 lifespan even beyond what has been seen with E. coli OP50, acting as a lifespan-extending bacterium when interacting with this host genetic background. More work is needed to identify the genetic differences between the two M. nematophilum strains and how lack of insulin host signalling modifies these bacterial strains and their properties.

The insulin pathway-mediated modification of a pathogen to a commensal (CBX102) or to a lifespan-extending bacterium (UV336) may have parallels in other model organisms. Recent evidence in mice has shown that inducing insulin resistance through dietary iron drove conversion of a pathogen to a commensal. Specifically, insulin resistance converted the enteric pathogen Citrobacter to a commensal (Sanchez et al, 2018). There, reduced intestinal glucose absorbance was crucial for Citrobacter to be a commensal (Sanchez et al, 2018). More work is needed to determine if systemic glucose levels and/or intestinal glucose absorption play a role also in C. elegans and how this relates to the worm insulin pathway. Reduced glucose levels increase lifespan in worms (Watts and Ristow, 2017). Reducing glycolysis has been shown to induce mitochondrial OXPHOS to generate a lifespan-extending reactive oxygen species (ROS) signal (Schulz et al, 2007) while increased levels have the opposite effect (Schulz et al, 2007; Zarse et al, 2012). Limitations in this comparison include the fact that our one bacterium microbiota is different than the complex one in mice. Moreover, the insulin pathway in worms and mammals may have differences in biochemical terms (reviewed in Watts and Ristow, 2017).
Taken together, our results and recent data from mice (Sanchez et al, 2018) show that the consequences a bacterium will cause to a host exist as a continuum. Thus, host genetics is important to determine where a bacterium may lie in this continuum. Our data show that interaction between the worm and its bacterial food will be shaped by both host genes as well as the bacterium at the strain level. In our system, the most prominent host proponent shaping this interaction is the insulin-FOXO-dependent signalling pathway. In this context, *C. elegans* is an excellent model to design genetic screens and identify worm mutants that suppress the UV336-dependent extension of the *daf-2* longevity phenotype.

**MATERIAL AND METHODS**

*C. elegans* strains: All strains (supplementary table S1) were provided by the *Caenorhabditis* Genetic Center (CGC), University of Minnesota, and maintained at 20 °C, unless otherwise noted. The CGC is supported by the National Institutes of Health – Office of Research Infrastructure Programs (P40 OD010440).

**Bacteria growth conditions:** *E. coli* OP50 or *M. nematophilum* (CBX102, UV336) cultures were grown in LB at 37 °C. Bacterial lawns were prepared by spreading 100 μl of an overnight culture on a 6 cm diameter NGM plate. Plates were incubated overnight at room temperature.

**Immunity and longevity Assays:** CBX102 assays were performed at 25 °C, unless otherwise noted, as previously described (Gravato-Nobre et al, 2016, Plos Pathogens). To test/validate immunity or longevity phenotypes of *daf-2* (e1370),
worms were raised on CBX102 or OP50 to the L4 stage at the permissive temperature (15 °C), and shifted to the restrictive temperature of 25 °C. Worms were age-synchronized by bleaching and embryos were incubated at 25 °C on NGM agar plates with lawns of *E. coli* OP50 or *M. nematophilum* CBX102. The embryonic stage (day of bleach) was designated as Day 0. A total of 125 worms were used per lifespan assay. On day 2, 25 animals were transferred to each NGM plate. Animals were scored daily and transferred to fresh lawns every other day. Death was defined when an animal no longer responded to touch. Worms that died of bagging or crawled off the plates were censored from the analysis. For each mutant population and bacterial lawn, the time required for 50% of the animal to die (TD50) was compared to that of the control populations using a *t* test. A *p*-value < 0.05 was considered significantly different from the control.

**SYTO 13 labelling:** Overnight bacterial cultures were concentrated 10x by spinning them at 2500 rpm, and their pellet suspended in 1 ml of TBS containing 3 μl of SYTO 13. Bacterial colonization was determined by exposing the animals to SYTO13-labelled CBX102 or OP50. To allow for their complete post-embryonic development, animals were left on CBX102 lawns, at 15 °C until most mutant animals reached L4, after which, they were shifted to 25 °C for another day. On day 7, one-day-old adult worms were exposed to SYTO 13-labelled CBX102. Worms were visualized after 20 hours of feeding on SYTO 13-labeled CBX102. Live worms were mounted on a glass slide in 25 μM tetramisole on a 2% agarose pad and examined using a Leica SP5 confocal microscope. Quantification of SYTO13 was performed in the intestine using a Leica TCS-SP5 Laser Scanning Confocal microscope using a x63 oil immersion lens and the Argon 488 laser. The focal plane with the highest GFP signal was used
to measure fluorescence intensity within a ROI set to 0.4 μ thickness, and a 10 μ or 40 μ diameter, for L1 larvae or adults, respectively. To make comparisons across samples, data are presented in boxes plots which define interquartile range (25% of the data above or below the median), bars represent expression range, and the thick line is the median. Identical exposure settings were used for all genotypes. Fluorescence was limited to 495/512 nm to diminish background autofluorescence from the animals. For each experiment, and on the same day, we imaged 10–15 animals per treatment.

**Thrashing Assays:** One-day old adults were placed in a drop of M9 and allowed to recover for 40 s (to avoid behaviour associated with stress), after which animals were video recorded for 30s. The number of body bend per second (BBPS) was determined by importing captured video images to ImageJ and by using wrMTrck plugin developed by Jesper S, Pederson. (http://www.phage.dk/plugins/wrmtrck.html). More than 20 animals were used in each treatment. Thrashing experiments were done in triplicates. All statistical analysis data performed using GraphPad Prism software.

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FIGURE 1. Lifespan, health and bacterial colonisation of the reference strain N2 in UV336 vs. CBX102. (A) Lifespan analysis at 25°C showing that CBX102 (red) significantly reduced average survival calculated using the Mantel-Cox log-rank test, 95% Confidence Interval (CI) compared to UV336 and OP50. The latter strains were statistically indistinguishable (NS). (B) Rigorous movement (thrashing) of animals grown on OP50, UV336 or CBX102 as a proxy for health was calculated as the number of body bends per second (BBPS). Tukey’s multiple comparisons with one-way ANOVA test was performed. Worms on CBX102 were significantly less mobile than on OP50 or UV336. These were again, statistically indistinguishable (NS). (C) Shown are distributions for the fluorescence intensity of SYTO13 in the intestine of animals on OP50 (E. coli), UV336 (M. nematophilum, non-inflammatory strain) and CBX102 (M. nematophilum pathogenic strain) at 25°C. Asterisks indicate the results of Two-Tukey’s multiple comparisons one-way ANOVA tests, 99% CI. All panels: ***p<0.0001, NS=non-significant and n=25 animals/treatments/group. Results are from 3 independent experiments.
FIGURE 2. Lifespan of *C. elegans* mutants define 4 groups on the pathogenic *M. nematophilum* strain CBX102. (A) Mutations that significantly shorten the lifespan compared to *ily*-3. TD50 =5 days. (B) Mutations that shorten the lifespan to the same degree as *ily*-3. (C) Mutations with the same TD50 as N2 (8-9 days). (D) Mutations that extended the average survival compared to N2 (e.g. *daf*-2=44 days). N=100 animals per survival curve.
FIGURE 3. The *daf-2* mutant modifies the effects on lifespan of *M. nematophilum* strains. (A) Lifespan of N2 on *M. nematophilum* CBX102 under CGI was significantly reduced (TD$_{50}$=11 days) when compared to both the derived *M. nematophilum* UV336 strain as well as *E. coli* OP50 that produced identical TD$_{50}$ (19 days). (B) Lifespan of *daf-2* on *M. nematophilum* CBX102 under CGI (TD$_{50}$=33) was statistically indistinguishable (p=0.4531) to OP50 (TD$_{50}$=36). In contrast, lifespan on UV336 was significantly (p<0.0001) increased (TD$_{50}$=49). For experiments involving the temperature sensitive *daf-2*, lifespan assays started at day 0 when animals were age-synchronized by bleach. Embryos were then left at 15°C on the appropriate bacterial diet till day 5. Day 5 marks the L4 to adult transition and time when plates were transferred to 25°C. The graph is A is the same experiment as in Fig 1A. All experiments shown in Figs 1 and 3 were conducted in parallel. N=25 per treatment. Results are from 3 independent experiments.
FIGURE 4. FOXO mediates the extension of *daf-2* lifespan on CBX102 under CGI. The *daf-2*-mediated lifespan extension on CBX102 was suppressed by *daf-16/FOXO*, using two mutants (*mu86* and *mgDf47*) of *daf-16*. We found that when compared to each other and to N2, both *daf-16, daf-2* double mutants as well as N2 had a lifespan with identical TD$_{50}$ (12 days) on CBX102. This was also the lifespan TD$_{50}$ of *daf-16(mu86)* alone (12 days). In contrast, lifespan of *daf-2* on CBX102 under CGI was significantly different (TD$_{50}$=33, p<0.0001). For experiments involving the temperature sensitive *daf-2*, lifespan assays started at day 0 when animals were age-synchronized by bleach. Embryos were then left at 15$^\circ$C on the appropriate bacterial diet till day 5. Day 5 marks the L4 to adult transition and time when plates were transferred to 25$^\circ$C. N=25 per treatment. Results are from 3 independent experiments.
| Group | Genotype   | CBX102 colonisation Day-2 adult | TD50 CBX102 25°C | p-value | Health CBX102 Day-1 adult (BBPS) | p-value |
|-------|------------|-------------------------------|------------------|---------|---------------------------------|---------|
| A*    | sek-1 (ag1)| +++                           | 5                | <0.0001 | 0.5194                          | 0.0001  |
|       | nsy-1 (ag3)| ++                            | 5                | <0.001  |                                 |         |
|       | pmk-1 (km25)|                           | 5                | <0.001  |                                 |         |
|       | lys-7 (ok1384)|                        | 5                | <0.0001 | 0.9708                          | 0.0001  |
|       | pmh-2 (ad597)|                        | 5                | <0.0001 |                                 |         |
|       | hsf-1 (sy411)|                        | 5                | <0.0001 |                                 |         |
| B*    | ilys-3 (ok3222)|                      |                   |         |                                 |         |
|       | bar-1 (nu63)| +++                          | 7                | 0.0166  |                                 |         |
|       | cep-1 (gk138)| +++                          | 7                | 0.0031  |                                 |         |
|       | dbl-1 (nk3)| ++                           | 7                | 0.9149  |                                 |         |
|       | vhl-1 (ok161)| +++                          | 7                | 0.006   |                                 |         |
|       | sur-2 (e2706)| ++                           | 8                | 0.9578  |                                 |         |
|       | eat-2 (ad465)| ++                           | 6                | 0.191   |                                 |         |
|       | kgb-1 (mu3)| +++                          | 7                | 0.7053  |                                 |         |
| C**   | N2 (wild type)|                    |                   |         |                                 |         |
|       | sqrt-3 (e24)| ++                            | 8                | 0.5673  |                                 |         |
|       | sqrt-3 (e2117)|                         | 9                | 0.5201  | 0.8253                          | 0.4641  |
|       | ced-1 (e1735)| ++                           | 8                | 0.0793  |                                 |         |
| D**   | daf-2 (e1370)| ++                           | 46               | <0.0001 | 1.261                           | 0.0001  |
|       | age-1 (hx546)| +                            | 10               | <0.01   | 1.332                           | 0.0001  |
|       | clk-1 (e2519)| +++                          | 14               | <0.0001 |                                 |         |
|       | hif-1| ++                            | 10               | 0.0094  |                                 |         |

**Table S1.** Statistics for Lifespan and Health span assays and mutants tested. For group categories see Fig. 2. WT is wild type (strain N2). Measurements: A* and B* relative to *ilys-3* and C** and D** relative to the reference strain (N2). C. elegans mutants without a numerical value in the health span column were not moving at all and therefore we were unable to film their vigour. All lifespan experiments above were done in parallel.
FIGURE S1. *M. nematophilum* CBX102 accelerates ageing. Representative images from animals expressing the mitochondria marker *mito-GFP* in the intestine (A), (C) in OP50 showing normal tubular mitochondria (arrows) while age-matched (B), (D) CBX102-grown Day-1 and Day-2 animals show fragmented mitochondria (arrows) with irregular shape. N=25 per treatment. Results are from 3 independent experiments.
FIGURE S2. Quantification of bacterial colonisation of CBX102 in *C. elegans* mutants.

Each dot represents a 1-day adult animal with SYTO13 fluorescence counted. *M. nematophilum* strain CBX102 displayed less colonisation in *daf-2* compared to N2 (designated as wild-type of WT). In contrast, mutants lacking the antimicrobial *ilys-3* gene, displayed significantly increased colonisation. Dunnett's multiple comparisons one-way ANOVA test was performed. ****P<0.0001; except comparisons with *ilys-3* and *daf-2*, all other comparisons were not significant.
**FIGURE S3. Tail swelling and colonisation when N2 and daf-2 grow in CBX102.**

Representative images of worms 10 days after bleaching of L1. In comparison to N2 daf-2 were found to have no tail swelling (arrows) and reduced intestinal colonisation. We looked at 25 worms per genotype per treatment in 3 independent experiments.
**FIGURE S4. Health of animals with different microbiota.** Box plot represents body bends per second (BBPS) counted per animal for each strain. Each box represents a group of 1-day adult animals (n=25). Dunnett’s-multiple comparisons one-way ANOVA test was performed showing that CBX102 was always significantly lower across the same host genotypes (p<0.0001) while *daf-2* was significantly higher than N2 across bacterial strains (p<0.0001). Comparison between *daf2* and *daf-16, daf-2* showed significant difference (p<0.0001) across the different bacteria while N2 and *daf-16, daf-2* were statistically indistinguishable (p>0.1).
FIGURE S5. Colonisation of OP50 and CBX102 in *daf-2*. Both bacterial species hardly colonised the gut of *daf-2* mutants opening up the possibility that these worms ate less. Representative images of worms 10 days after bleaching of L1. We looked at 25 worms per genotype per treatment in 3 independent experiments.