Role of polyunsaturated fatty acids during infection of *Caenorhabditis elegans*

By

Nthabiseng Zelda Mokoena

Submitted in accordance with the requirements for the degree

Philosophiae Doctor in Microbiology

in the

Department of Microbiology and Biochemistry

Faculty of Natural and Agricultural Science

The University of the Free State

Study Leader: Prof. C. H Pohl-Albertyn

Co-study leader: Prof. J Albertyn

April 2022

Declaration

I, Nthabiseng Zelda Mokoena declare that the Doctoral dissertation that I herewith submit at the University of the Free State, is my independent work and that I have not previously submitted it for qualification at another institution of higher education.

I, Nthabiseng Zelda Mokoena declare that I am aware that the copyright is vested in the University of the Free State.

I, Nthabiseng Zelda Mokoena declare that all royalties as regards to intellectual property that was developed during the course of and/or in connection with the study at the University of the Free State, will accrue to the University.

I, Nthabiseng Zelda Mokoena declare that I am aware that the research may only be published with the Dean's approval.

NZ Mokoena 14 Apr 2022

Signature Date

Acknowledgements

- Prof CH Pohl Albertyn - Main supervisor
- Prof J Albertyn - Co-supervisor
- Pathogenic Research Group
- The Department of Microbiology and Biochemistry
- Prof A Hugo for assistance with GC-analysis
- Dr G Kemp for assistance with the LC-MS/MS
- Mr Sarel for assistance with GC-analysis
- Dr Caroline Dickens, Therese Dix-Peek and Christelle Klopper for assistance with Nanostring
- Dr Errol Cason for assistance with Venn diagrams
- *Caenorhabditis elegans glp-4; sek-1* hermaphrodite strain was provided by the Caenorhabditis Genome Centre, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Financial assistance:

- National Research Foundation (NRF) is hereby acknowledged for their financial assistance towards this research project.

Personal acknowledgements

- Prof CH Pohl Albertyn; The most amazing supervisor, her support, patience and guidance has contributed a lot to this research project. Her mentorship has shaped and moulded me in an amazing way. I am truly thankful for all her contributions towards my PhD journey.
- Gratitude goes to my mother (Majoase Mokoena) and father (Tello Muzi Mlangeni)
- Family for all the love and support
- Friends and loved ones for all the encouragement and support
- Dr T Motaung for his guidance and motivation during my PhD journey
- Pastors and all Saints of God for their prayers

With God all things are possible

Table of Contents

Chapter 3

The influence of long-chain polyunsaturated fatty acids on behaviour, susceptibility to infection and egg retention in *Caenorhabditis elegans*

Chapter 4

Exploring the mechanism behind the effect of EPA supplementation on *C. albicans* infection

---

Chapter 5

General discussions and conclusions

Supplementary documents

Dissertation summary

During polymicrobial infection, interactions between different microbial species can alter host responses and/or microbial virulence and pathogenesis, often complicating patient treatment and resolution of infection. Polyunsaturated fatty acids (PUFAs) are not only crucial for normal function in mammalian systems, but are also proposed to act as endogenous antimicrobial molecules. Thus, we adopted *Caenorhabditis elegans* to mimic the *Pseudomonas aeruginosa* and *Candida albicans* polymicrobial infections found in humans. We determined the influence of arachidonic acid (AA) and eicosopentaenoic acid (EPA) supplementation on mono- and polymicrobial infection, fatty acid (FA) composition and egg retention of the nematodes, as well as on expression of FA metabolic genes. Supplementation with either AA or EPA in mono- and polymicrobial infections resulted in changes in FA profiles and PUFA biosynthesis pathway. We discovered that the degree of egg retention elicited by *C. albicans and P. aeruginosa* varied, with *C. elegans* exposed to both *C. albicans and P. aeruginosa* showing the highest level of egg retention compared to nematodes infected by either *C. albicans or P. aeruginosa* alone. Interestingly, the AA supplemented infected nematodes showed an increased level of egg retention, while EPA supplemented infected nematodes showed a significant decrease. Using the *C. elegans* model, we determined the effects of AA and EPA supplementation on the survival of nematodes with mono- and polymicrobial infection. We showed that the survival of the infected nematodes was influenced by PUFA-supplementation. EPA supplementation effectively reduced *C. albicans* virulence and inhibited hyphal formation, thus leading to a partial rescue of pathogen susceptibility. However, this was not the case for *P. aeruginosa* infections. In fact, EPA supplemented nematodes infected with *P. aeruginosa* were more susceptible. Notably hyphal formation is an important component of *Candida* pathogenesis in mammals. Furthermore, polymicrobial infection resulted in synergistic virulence. However, *C. albicans* did not produce any hyphae in the co-infection of either AA, EPA supplemented or unsupplemented nematodes, this suggests that the increase in pathogenesis may be associated with increased *P. aeruginosa* pathogenesis. To further test the role of EPA in hyphal formation of *C. albicans*, we hypothesised that cytochrome P450 (CYP450) metabolises EPA to 17,18-epoxyeicosatetraenoic acid (17,18-EpETE), inhibiting *C. albicans* hyphal formation. We showed that 17,18-EpETE inhibits *C. albicans* hyphal formation *in vitro* and *in vivo* in *C. elegans* and that inhibitors of mammalian EPA-metabolising CYP450 enzymes, 17-octadecynoic acid (17-ODYA) and 6-(2-propargyloxyphenyl)hexanoic acid (PPOH) restored *C. albicans* hyphal formation *in vivo*. Lastly, the role of EPA on the physiology of *C. elegans* as well as *C. albicans in vivo* was investigated using gene expression analyses. Among the up-regulated genes, we observed several genes with potential roles in lipid metabolism, hyphal formation, detoxification, stress response and immune response. For

instance, we observed an up-regulation of several involved in the synthesis of FAs, including *fat-3*, *fat-4*, *fat-6*, *cyp-29A2* and *cyp-37A1.* Other up-regulated genes were those involved in immune response, such as *cyp-37B1*, *daf-16*, *fipr-22*, *ilys-2*, *lys-5*, *lys-6*, *spp-12* and *fat-3*. Interestingly common genes involved in hyphal formation, such as *CAS5*, *CRZ1*, *CTA4*, *ERG11*, *FCR1*, *SNQ2*, *TAC1*, *TEC1*, *YOR1* and *ZCF3* were also up-regulated. Overall, the benefits of EPA supplementation may be two-fold, by inhibiting virulence factors of *C. albicans* and stimulating the immune response of the host. Thus, PUFA supplementation might be useful in the treatment of infections in patients caused by *C. albicans* and *P. aeruginosa*.

Chapter 1

Literature review

1. Section A

1.1. Motivation

Infectious diseases have been recognized as one of the global burdens that largely affect the world economy. An infection is not a simple two-way interaction between a pathogen and the host, but rather includes the interaction with other pathogens colonizing mucosal surfaces. For instance, opportunistic pathogens *Candida albicans* and *Pseudomonas aeruginosa* can form complex communities in a variety of niches within the human body in both healthy individuals and in the event of disease (Peleg *et al*., 2008; Bergeron *et al*., 2017). *Candida albicans* and *P. aeruginosa* are often co-isolated at various infection sites, pointing to mixed infections by the two, including patient wounds, contaminated catheters (vascular and urinary catheters), the sputum of cystic fibrosis patients, bronchial airways and lung infections, and they influence each other's virulence potential (Hughes and Kim, 1973; Bauernfeind *et al*., 1987; Hermann *et al*., 1999; de Macedo and Santos, 2005). Under normal or disease-free conditions, both species are commensals of healthy individuals. However, in a diseased state where an individual's defences are supressed, the pathogens become invasive, resulting in serious disease and, ultimately, death (Naglik *et al*., 2004; Hube, 2006; Pfaller and Diekema, 2007). The plethora of virulence mechanisms exploited by *C. albicans* account for why this pathogen is well-adapted to the human host. Its dimorphism, for example, makes it a morphologically flexible fungus that can colonize the host either in the yeast or filamentous form (Chandra *et al*., 2001; Lohse *et al*., 2018). In response to certain stimuli (e.g., signaling molecules, temperature, and host factors), it switches from yeast form to hyphal form (Gow, 1997; Calderone and Fonzi, 2001; Gow *et al*., 2002; Liu, 2002; Whiteway and Oberholzer, 2004). The ability to transition between yeast and filamentous forms have been shown to be an important virulence factor during *C. albicans* infection, the yeast form drives dissemination while filamentous form are primarily invasive (Chandra *et al*., 2001; Gow *et al*., 2002; Douglas, 2003). Interestingly, several studies have suggested that *P. aeruginosa* can influence fungal infections by inhibiting growth of *C. albicans* and eliminating the fungus from the host (Bauernfeind *et al*., 1987; Kerr, 1994; Burns *et al*., 1999; Gupta *et al*., 2005; Kaleli *et al*., 2007; Oever and Netea, 2014). However, the interplay between *C. albicans* and co-localised *P. aeruginosa* does not always have a beneficial effect on the host, but can also result in an enhanced virulence of either the fungal or the bacterial species (Neely *et al*., 1986; Roux *et al*., 2009; Diaz *et al*., 2012; Xu *et al*., 2014; Bergeron *et al*., 2017). The interaction between bacteria and fungi can exert effects on microbial behaviour, dissemination, survival, the response to antimicrobials and, ultimately, patient prognosis, which often complicates patient treatment and resolution of infection (Hogan and Kolter, 2002; Kerr, 1994; McAlester *et al*.,

2008; Méar *et al*., 2013; Lindsay and Hogan, 2014; Trejo-Hernández *et al*., 2014). Moreover, *C. albicans* and co-localised *P. aeruginosa* form biofilms with increased resistance to antimicrobial agents. A significant amount of research has thus focused on gaining an understanding of how inter-domain interactions affect biofilm formation and the response to antimicrobial therapies (Harriot and Noverr, 2009; Peters *et al*., 2012; Murray *et al*., 2014).

Over the years, in order to circumvent problems associated with antimicrobial resistance, the search for new antimicrobials, such as fatty acids (FAs), have gained momentum due to their antibacterial, antifungal and antiviral properties (Kohn *et al*., 1980; Pohl *et al*., 2011; Yoon *et al*., 2018). Polyunsaturated fatty acids (PUFAs) exhibit a diverse range of important biological functions in most biological systems, including acting as endogenous antimicrobial molecules (Watts *et al*., 2003; Deline *et al*., 2013; Tallima and El Ridi, 2018; Das, 2018). These PUFAs can be oxygenated via enzymatic or free radical-mediated reactions to form bioactive oxygenated lipid mediators termed oxylipins (Funk, 2001; Catalá, 2010; Stables and Gilroy, 2011; Massey and Nicolaou, 2011; Vrablik and Watts, 2013). Eicosanoids are broad class of oxylipins that are transient and locally synthesized signaling molecules, including prostaglandins, leukotrienes, lipoxins and thromboxanes, which mediate various physiological responses, such as inflammation (Noverr *et al*., 2001; Erb-Downward and Huffnagle, 2007). Hence, diverse vertebrate and non-vertebrate model organisms including *Mus musculus*, *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Danio rerio* and Arabidopsis have been employed to screen, not just the virulence factors and to decipher the common virulence mechanism by pathogens, but to also screen potential antimicrobials (Gratacap *et al*., 2014; Bergeron *et al*., 2017). Testing pathogenesis and immune defence in rodents are generally not feasible due to its expensive maintenance. Thus, the invertebrate nematode model *C. elegans* could represent a valuable alternative. Since *C. elegans* has a well-defined anatomy, short life cycle, genetic similarity to the human genome and range of available genetic tools (Brenner, 1974; Corsi *et al*., 2015), it has been widely used to study functions of PUFAs (Watts, 2016). The nematode *C. elegans* synthesizes all of the enzymes necessary to produce a range of omega-6 (n-6) and omega-3 (n-3) FAs and many of the long chain PUFAs found in humans (Wallis *et al*., 2002) and represents a good model organism to systematically study the antimicrobial roles of these molecules (Kong *et al*., 2016). The ability to alter FA composition with genetic manipulation and dietary supplementation permits the dissection of the roles of n-3 and n-6 FAs in many biological process including reproduction, aging, and neurobiology (Watts and Browse, 2002; Watts *et al*., 2003; Brock *et al*., 2006; Deline *et al*., 2013; Yi *et al*., 2014; Anderson *et al*., 2019; Jia *et al*., 2019). Studies in *C. elegans* to date have mostly identified overlapping functions of 20-carbon n-6 and n-3 FAs in reproduction and in neurons (Vrablik and Watts, 2013; Watts, 2016). However, specific roles

for either n-3 or n-6 FAs in pathogenesis are beginning to emerge. Taken together, these studies are an addition to the currently established health-promoting effects of monounsaturated fatty acids (MUFAs) (Anderson *et al*., 2019), thus suggesting an ancient link between metabolism, nutrient storages and susceptibility of the host to bacterial infection. The resistance of *C. albicans* and *P. aeruginosa* to conventional antimicrobial treatment is a major scourge in healthcare (Wolcott *et al*., 2013; Stacy *et al*., 2016; Costa-Orlandi *et al*., 2017; Kim *et al*., 2018). To face this global crisis by infectious diseases, the interaction between host and infectious agents has to be understood. Therefore, it is crucial that novel potent anti-infectives are discovered. Taking into consideration the increasing importance of *C. albicans* and *P. aeruginosa* towards morbidity and mortality of most hospitalized patients and uprising antimicrobial resistance. The aim of the present study was to further define *C. albicans* and *P. aeruginosa* pathogenicity and to investigate the role of PUFAs on their virulence using a non-mammalian host model *C. elegans*. An in-depth understanding of these interactions could be exploited for the benefit of mankind.

2. Section B

Section B of chapter 1 is a review article published in *Prostaglandins* and *Other Lipid Mediators* journal, following the reference style of the journal.

The candidate, Nthabiseng Zelda Mokoena, wrote the manuscript and designed the figures, Olihile Sebolai and Jacobus Albertyn edited the manuscripts, while Carolina H. Pohl supervised and edited the final manuscript.

Citation

Mokoena, N. Z., Sebolai, O. M., Albertyn, J. & Pohl, C. H. (2020). Synthesis and function of fatty acids and oxylipins, with a focus on *Caenorhabditis elegans. Prostag Oth Lipid M* **148**, 106426. doi.org/10.1016/j.prostaglandins.2020.106426

Prostaglandins and Other Lipid Mediators 148 (2020) 106426

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

journal homepage: www.elsevier.com/locate/prostaglandins

Synthesis and function of fatty acids and oxylipins, with a focus on *Caenorhabditis elegans*

N.Z. Mokoena, O.M. Sebolai, J. Albertyn, C.H. Pohl*

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, Bloemfontein, South Africa

ARTICLE INFO

Keywords:
Polyunsaturated fatty acids
Oxylipins
Eicosanoids
Arachidonic acid
Caenorhabditis elegans

ABSTRACT

Polyunsaturated fatty acids (PUFAs) exhibit a diverse range of important biological functions in most biological systems. These PUFAs can be oxygenated via enzymatic or free radical-mediated reactions to form bioactive oxygenated lipid mediators termed oxylipins. Eicosanoids are broad class of oxylipins that are transient and locally synthesized signalling molecules, including prostaglandins, leukotrienes, lipoxins and thromboxanes, which mediate various physiological responses, such as inflammation. In addition to arachidonic acid-derived eicosanoids, current developments in lipidomic methodologies have brought attention to vast number of oxylipins produced from other PUFAs, including omega-3. Although, the molecular mechanisms of how PUFAs and oxylipins contribute to majority of the fundamental biological processes are largely unclear, a model organism *Caenorhabditis elegans* remains a powerful model for exploring lipid metabolism and functions of PUFAs and oxylipins. For instance, the ability of *C. elegans* to modify fatty acid composition with dietary supplementation and genetic manipulation enables the dissection of the roles of omega-3 and omega-6 PUFAs in many biological processes that include aging, reproduction, and neurobiology. However, much remains to be elucidated concerning the roles of oxylipins, but thus far, *C. elegans* is well-known for the synthesis of vast set of cytochrome (CYP) eicosanoids. These CYP eicosanoids are extremely susceptible to changes in the relative bioavailability of the different PUFAs, thus providing a better insight into complex mechanisms connecting essential dietary fatty acids to various biological processes. Therefore, this review provides an overview of the synthesis and function of PUFAs and oxylipins in mammals. It also focusses on what is known regarding the production of PUFAs and oxylipins in *C. elegans* and their functions.

1. Introduction

The roles of lipids can be observed across all domains in biology since lipids play important roles as either building blocks of membranes, efficient source of energy or as messengers during cellular signal transduction [1,2]. Polyunsaturated fatty acids (PUFAs) are precursors of numerous lipid metabolites with potent bioactivities, produced via enzymatic and/or free radical-mediated reactions [3–7]. For instance, in mammals, oxygenated PUFAs that are enzymatically derived are produced via three main pathways, namely cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome (CYP) P450 pathways, which produce wide range of metabolites, such as eicosanoids, octadecanoids and docosanoids [3,7–12]. Eicosanoids are well studied families of bioactive lipids, derived from carbon-20 (C20) PUFAs, such as arachidonic acid (AA; 20:4n-6), dihomo-α-linolenic acid (DGLA; 20:3n-6) and eicosapentaenoic acid (EPA; 20:5n-3) [3,7,11,13]. Arachidonic acid-derived eicosanoids have been shown to have pivotal role in inflammation, primarily being pro-inflammatory, in contrast EPA- and DGLA-derived eicosanoids are considered to dampen down or oppose this effect, thus shifting the biological setting to become anti-inflammatory [13–18,2].

Nematode *Caenorhabditis elegans* is emerging as key biological system to study lipid functions in whole organisms [19]. It is considered a powerful tool due to numerous features, such as a well-annotated genome, simple anatomy, short lifespan, ease of genetic analysis and well-understood developmental programs allowing diverse studies of biological processes [20]. A unique aspect of *C. elegans* fatty acid biosynthesize is the ability to synthesize a wide range of PUFAs *de novo* [21,2]. Unlike other animal species, *C. elegans* harbours complete set of genes required for biosynthesis of n-3 and n-6 PUFAs, such as AA, EPA and other related PUFAs, which are only accessible to mammalian organisms via nutrition [7,21–24]. The *C. elegans* PUFA mutants strains display many growth, reproduction, mechanosensory, olfactory and osmosensory deficits, neuromuscular defects, and reduced brood size

* Corresponding author.
E-mail address: PohlCH@ufs.ac.za (C.H. Pohl).

https://doi.org/10.1016/j.prostaglandins.2020.106426
Received 18 July 2019; Received in revised form 24 January 2020; Accepted 31 January 2020
Available online 04 February 2020
1098-8823/

that can be rescued by feeding with C20-PUFA [21,25–29]. In addition, PUFAs are essential for signalling found in oocytes that facilitates the recruitment of sperm to spermatheca [30,31]. Fascinatingly, *C. elegans* does not possess any clearly identifiable orthologues of mammalian COX and LOX enzymes nor receptors of prostanoid and leukotriene [11,26,32]. However, the nematode possess several CYP P450 genes which are homologs of mammalian CYP isoforms significant for synthesis of AA metabolites [11,33,34]. Therefore, since oxylipins are found in most biological milieus, and are formed through different pathways and substrates, it is important to address questions regarding their synthesis and function using appropriate biological models. Here we provide a broad overview of the similarities and differences of synthesis and regulation of PUFAs and oxylipins in mammals and compare it to a facile model host, *C. elegans*. Previous studies have reported that *C. elegans* share evolutionarily conserved fatty acid biosynthesis pathway with mammals, thus it enables manipulations in order to understand fatty acid synthesis in mammals, since vertebrate models are more difficult to manipulate. Next, we explore the functions of PUFAs and oxylipins in both mammals and *C. elegans*. Since *C. elegans* share lipid metabolism pathways with mammals, understanding the functions of *C. elegans* PUFAs and oxylipins will potentially assist in linking the functions of PUFAs and oxylipins in *C. elegans* to those in mammals.

2. Oxylipin synthesis in mammals

Depending on the double bond (n) position in close proximity to the methyl end of the fatty acid chain, PUFAs can be categorised as either omega-3 (n-3) or omega-6 (n-6) fatty acids [35]. In mammals, n-3 and n-6 PUFA families that are 18-carbon and longer cannot be synthesized *de novo*, thus produced from the dietary essential fatty acids [α-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6)], through a chain of desaturation and elongation reactions that are catalysed by various desaturase and elongase enzymes [18,35–37,2]. Thereafter, C20-PUFAs can synthesize oxylipins via mono- or dioxygen-dependent reactions, with eicosanoids produced from AA, being the most predominant oxylipin. This AA can be synthesized from LA, which is mostly found in vegetable oils or directly accessible from dairy and meat products (Fig. 1) [38,39,2]. Oxylipins can also be synthesized from other PUFAs, commonly octadecanoids formed from LA and ALA, eicosanoids formed from DGLA and EPA, and also docosanoids formed from adrenic acid (AdA; 22:4n-6) and docosahexaenoic acid (DHA; 22:6n-3) [13,18,36,2]. For instance EPA and DHA can be synthesized from dietary ALA (mostly abundant in walnuts and leafy green vegetables), however, this conversion is very limited in mammals [40–42]. Moreover, the biosynthesis pathway is regulated by highly polymorphic desaturase and elongase genes, and also controlled by sex hormones, which results into large interindividual differences in the efficiencies of ALA synthesizing EPA and DHA [40,42]. Furthermore, at all steps of this pathway, n-6 and n-3 PUFAs are known to largely compete for desaturation and elongation. Thus, the most predictable and efficient way to supply the demand for EPA and DHA is to directly supplement them with these long-chain n-3 PUFAs.

Oxylipin synthesis begins with cell activation, resulting into the liberation of the PUFAs precursors in the phospholipids membrane sn-2 position by cytosolic phospholipase A_2 (cPLA$_2$) (Fig. 2) [43–45]. These PUFAs are then metabolized into oxylipins by one of the three pathways: cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome (CYP) P450 pathways [3,9,11,12,46–51]. In cases where the C20-PUFA is metabolized into oxylipins via COX isoforms (for instance, the constitutive COX-1 or inducible COX-2), and also subject to terminal synthases, the end products produced are usually prostacyclin, thromboxanes (TXs) and prostaglandins (PGs), collectively termed prostanoids (Fig. 2) [3,9,10,49,51]. For example, the COX can convert various PUFAs, such as DGLA, EPA, AA, and AdA into 1-, 2-, 3- and dihomo-2-series prostanoids, such as prostaglandin D_1 (PGD_1), PGD_2, PGD_3, and

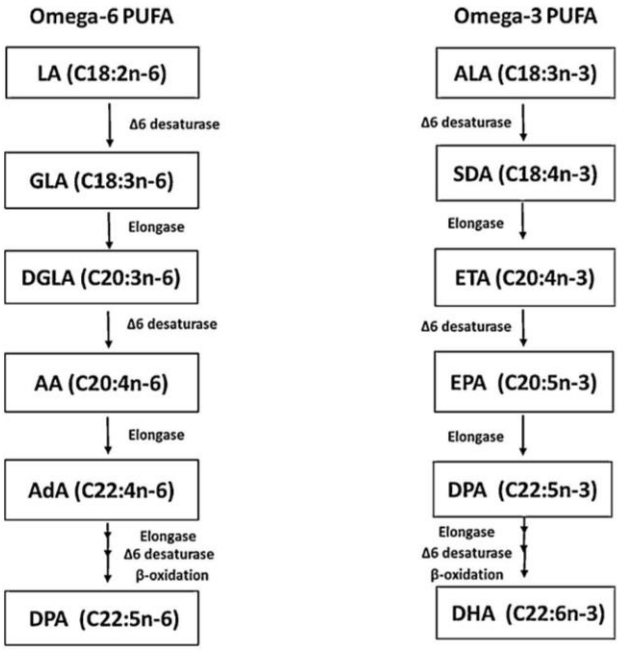


Fig. 1. Long-chain n-6 and n-3 polyunsaturated fatty acids (PUFAs) biosynthesis pathway in mammals. Linoleic acid (LA; 18:2n-6) serve as a precursor of arachidonic acid (AA; 20:4n-6) and adrenic acid (AdA; 22:4n-6), while α-Linolenic acid (ALA; 18:3n-3) may serve as a precursor of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). The biosynthesis pathway is regulated by highly polymorphic desaturase and elongase enzymes as indicated with arrows.

dihomo-PGD_2 [9,11,52]. Moreover, COX can also form certain hydroxy fatty acids, for example 9-hydroxy-octadecadienoic acid (9−HODE) from LA, 11-hydroxy-eicosatetraenoic acid (11-HETE) from AA, and 13-hydroxy-docosahexaenoic acid (13-HDoHE) from DHA [53,54]. While in the second pathway, lipoxygenases (LOXs) catalyse the synthesis of most hydroxy fatty acids and their metabolites, such as leukotrienes, resolvins, lipoxins, protectins, hepoxilins, maresins, and eoxins (Fig. 2) [9,10]. When PUFAs are subjected to LOX oxygenation, they can form a wide range of mono- and polyhydroxy fatty acids, for instance, AA forms leukotrienes (LTs), lipoxins (LXs) and hydroxy eicosatetraenoic acids (HETEs), while EPA forms E-series resolvins (RvEs) and hydroxy eicosapentaenoic acids (HEPEs). On the other hand, DHA forms various docosanoids, including D-series resolvins (RvDs), protectins (PDs), hydroxy-docosahexaenoic acids (HDHAs) and maresins, while LA produces octadecanoids [e.g. hydroxy octadecadienoic acids (HODEs)], and DGLA produces hydroxy eicosatrienoic acids (HETrE's) [9,10,55–58,2]. Interestingly, as LOX activities oxygenate AA, they can be defined by their positional selectivity, for example as 5-LOX, 8-LOX, 12-LOX, and 15-LOX. An example of this is the 5-LOX activity which forms 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) and 5-hydroxyeicosatetraenoic acids (5-HETE) from AA. Moreover, hydroxy fatty acids (5-HETE), formed through LOX, can further be metabolized to their dihydroxy derivatives [e.g., 5,15-dihydroxy-eicosatetraenoic acid (5,15-DiHETE)] or keto [(e.g., oxo-eicosatetraenoic acid (oxo-ETE)]. When 5-lipoxygenase activating protein activates 5-LOX, it forms leukotrienes, such as leukotriene B_4, and the cysteinyl leukotrienes [59]. Interestingly, combinations of sequential activities of LOX, which includes epoxygenase and hydrolase activities, are known to produce di- and tri-hydroxy fatty acids, such as resolvins, lipoxins, maresins and protectins [9,55,60]. While hepoxilins are produced from 12-hydroperoxy-eicosatetraenoic acid (12-HpETE), and eoxins are produced from 15-hydroperoxy-eicosatetraenoic acid (15-HpETE) [9,61,62]. An additional pathway that converts PUFA metabolism to oxylipins is

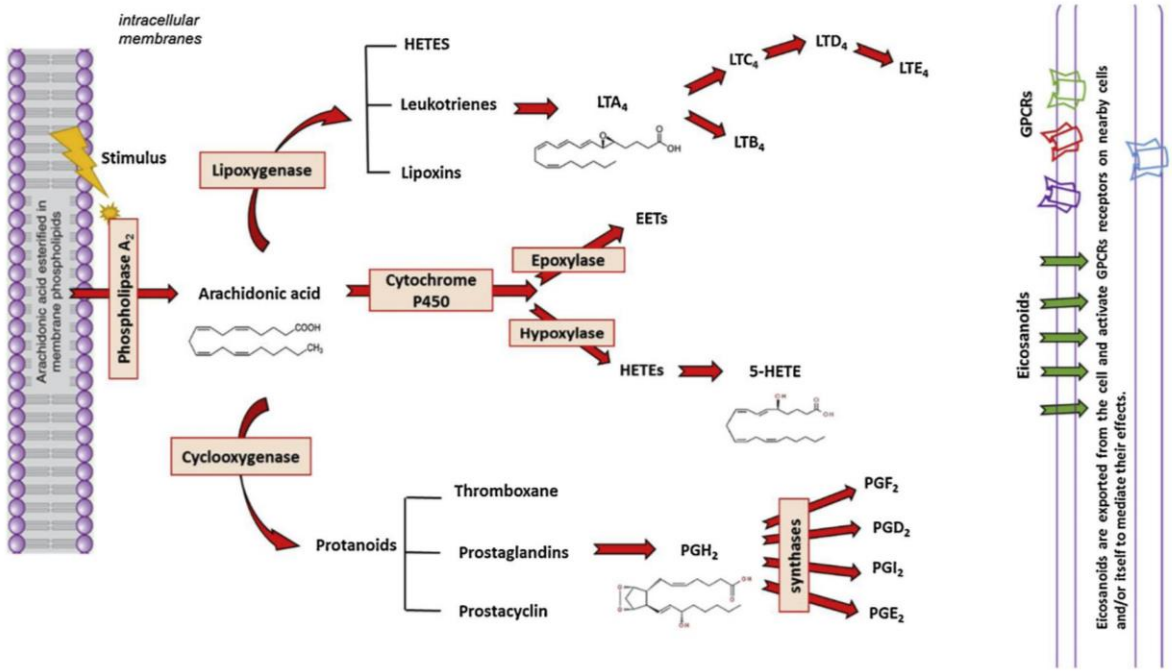


Fig. 2. Eicosanoids biosynthesis pathway. Stimulus triggers phospholipase A_2 (PLA_2) to translocate to intracellular membranes, where PLA_2 cleaves and releases arachidonic acid (AA). Cyclooxygenase enzymes (COX) catalyses AA to form prostacyclin, thromboxanes (TXs) and prostaglandins (PGs), collectively termed prostanoids. For prostaglandin synthesis, COX create the cyclic intermediate prostaglandin H_2 (PGH_2), which can further be synthesized into a series of prostaglandins [(prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2) prostaglandin I_2 (PGI_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)] through the activity of synthase enzyme. Alternately, AA can either be processed by lipoxygenase 5 (5-LOX) for synthesis of leukotrienes (LTs), lipoxins (LXs) and hydroxy eicosatetraenoic acids (HETEs), or processed by cytochromes P450 (CYP450) enzymes to form other linear oxygenated fatty acids through either epoxygenase or hydroxylase activity. For instance, epoxygenase activity produces epoxy-eicosatrienoic acids (EETs), while hydroxylase activity produces HETEs. These eicosanoids are then exported from the cell out and activates G-protein coupled receptors (GPCRs) on nearby cells or mediate their own effects.

catalysed by a wide range of cytochrome (CYP) P450 enzymes that can have either epoxygenase or ω-hydroxylase activity (Fig. 2) [9–12]. For example, CYP450 can convert AA, EPA, and DHA into epoxy-eicosatetraenoic acid (EpETE), epoxy-eicosatrienoic acid (EpETrE), and epoxy-docosapentaenoic acid (EpDPE or EDP) via epoxygenase activity, and also into HETE, HDoHE and hydroxy-eicosapentaenoic acid (HEPE) via ω-hydroxylase activity [9]. Furthermore, these epoxygenase products can be rapidly metabolized via soluble epoxide hydrolase (sEH) to produce dihydroxy fatty acids such as dihydroxy-eicosatrienoic acid (DiHETrE), dihydroxy-docosapentaenoic acid and DiHETE [9].

3. Oxylipin functions in mammals

Oxylipins display a broad range of functions, whereby majority are still being discovered. Fascinatedly, oxylipins derived from different substrate of PUFAs and from different pathways, can either possess similar or opposing effects, thus important to fully identify the overall oxylipin profile, for a proper understanding of their overall biological effects [13]. As stated above, in mammals, n-6 PUFAs, such as AA produce eicosanoids via the action of LOX and COX enzymes [11,63]. Therefore, these eicosanoids can either exert stimulatory or inhibitory influence, moreover they can have profound effects on numerous aspects of mammalian physiology. Their wide range of functions includes tissue repair, apoptosis, cell proliferation, pain, inflammation, blood clotting, blood vessel permeability, blood pressure regulation and immune actions [3,8,18,43]. In addition, eicosanoids, such as leukotrienes and prostaglandins, are known as pro-inflammatory mediators that play an important role as primary containments of an infection, and also for the recruitment of immune cells, including phagocytes, to a site of infection, while the n-3 fatty acids EPA demonstrate strong anti-inflammatory effects, and can influence the response of T cell to infection [64,65]. Moreover, due to humans lacking the ability to synthesize the long-chain PUFAs, which act as precursors of eicosanoids, they have become an integral part of their diet. Thus consuming food rich in PUFAs is associated with various health benefits, such as improved metabolism, longevity and function [18,66]. For instance, it has been suggested that n-3 PUFAs found in fish and other enriched food products can assist in the prevention of vascular dysfunction by decreasing vasoconstrictors [67]. Furthermore, in the cardiovascular system, n-3 EPA and DHA, have been demonstrated to have antithrombotic, anti-inflammatory and anti-arrhythmic properties [18,68]. However, a consensus view is still emerging regarding the precise targets and mechanisms by which majority of n-3 PUFAs exert their physiological functions in mammals. Some studies have shown that dietary fatty acids and eicosanoids can also bind nuclear receptors, including peroxisome proliferator activated receptor c, that function by modulating activation of T cells, NK cells and dendritic cells [69,70]. Furthermore, in mammals, oxylipins serve as second messengers of several growth factors, hormones, and cytokines that are important for regulation of cardiac, vascular, and renal function [63]. On the other hand, imbalances in CYP-eicosanoid production are mostly associated to the development of inflammatory disorders, cardiovascular disease, and cancer [63].

4. PUFA synthesis in *C. elegans*

In mammals, lipid signalling has become very complex and fine-tuned with diverse lipids possessing multiple functions in different organ systems, thus complicating our understanding of fatty acid signalling during most biological processes, including development and reproduction. Simple invertebrate model *Caenorhabditis elegans* has become ideal for discovering new functions and regulation of lipid metabolism due to its simple anatomy, and also wide range of molecular, forward and reverse genetic and whole organism tools [71,72,2]. Unlike humans that store lipids in dedicated adipose tissue, *C. elegans*

use intestinal and hypodermal cells to store lipids, however most aspects of lipid metabolism regulation are closely related to humans [73]. Despite these differences [74], identified many genes in an RNAi screen that altered fat storage, which includes homologues that play a role in mammalian fat metabolism. In addition, many pathways like insulin-signalling pathways are able to regulate fat storage not only in mammals but also nematodes [75,23].

Caenorhabditis elegans can directly absorb and incorporate dietary fats, and this enables researchers to modify the composition of fatty acids of live nematodes [21,2]. These fatty acids may also be modified by elongation and/or desaturation [21,2]. Moreover, the nematode can inherit fatty acids by maternal inheritance as well as synthesize a wide range of fatty acids *de novo* [76,2]. In mammals, acyl transferase enzymes prefers monounsaturated fatty acids (MUFAs), like oleic acid (OA; 18:1n-9) as substrate to synthesize triglyceride fatty acids [77]. However, in *C. elegans*, triglycerides mainly consist of dietary fatty acids obtained from *Escherichia coli* [77]. Similar to mammals, *C. elegans* possesses acetyl CoA carboxylase and fatty acid synthase, functioning as key enzymes for fatty acid biosynthesis [19,78]. At present several enzymes are shown to have a significant function during PUFA biosynthesis in *C. elegans*, such as seven fatty acid desaturase enzymes (*fat-1* to *fat-7*), one 3-ketoacyl-CoA reductase (*let-767*) and fatty acid elongases (*elo-1* and *elo-2*) (Fig. 3) ([21,22,27,79,80,2,24]. For instance, elongation is procured by specific elongase enzymes encoded by the *elo* genes (*elo-1*, *elo-2*, *elo-5* and *elo-6*) which facilitates the elongation of both saturated and unsaturated fatty acids [23]. Moreover, the nematode has genes for protein products mediating the endogenous synthesis of n-3 long-chain PUFAs, including *fat-1* gene, encoding n-3 desaturase (Δ15 desaturase) that converts n-6 LA and γ-linolenic acid (GLA; 18:3n-6) to n-3 ALA and stearidonic acid (SDA; 18:4n-3) [22]. Another unique fatty acid biosynthesis gene in *C. elegans* is *fat-2*, encoding the Δ12 enzyme, which facilitates the biosynthesis of n-6 LA from its substrate, n-9 OA [81,82]. The other genes, like *fat-3* gene, encoding Δ6 desaturase and *fat-4* gene encoding Δ5 desaturase, are involved in the biosynthesis of C20-PUFAs. As in mammals, *C. elegans* also possess Δ9 fatty acid desaturases, encoded by the three genes, *fat-5*, *fat-6* and *fat-7*, which produce MUFAs from saturated fatty acids (SFA) [21]. The FAT-5 desaturase particularly acts on palmitic acid (PAL; 16:0) producing palmitoleic acid (POA; 16:1n-7), which can further be elongated to *cis*-vaccenic acid (cVA; 18:1n-7) [83]. The FAT-6 and FAT-7 desaturases act on stearic acid (STE; 18:0) synthesizing OA [83]. Unlike in mammals, OA can further be desaturated and elongated to produce PUFAs in *C. elegans*, thus accumulated in a small degree in membranes of *C. elegans* and neutral lipids, despite it being a significant component found in mammalian membranes [37].

Briefly, the unsaturated fatty acid synthesis pathway in *C. elegans* begins with a precursor, PAL, which is either synthesized by acetyl CoA carboxylase and fatty acid synthase *de novo* or obtained from the *E. coli* diet [77,84]. Thereafter, PAL is catalysed by FAT-5 Δ9 fatty acid desaturase to form POA (Fig. 3) [21,24]. Then POA is elongated to cVA, which is the most abundant fatty acid in phospholipids and triglycerides [85]. The PAL can also be elongated to STE, the substrate for FAT-6 and FAT-7 desaturation to OA by Δ9 fatty acid desaturase [21,24]. Both cVA and OA are 18-carbon MUFAs, however they are metabolized differently in the nematode [21,22,86]. For instance, OA acts as a precursor and is catalysed by FAT-2 Δ12 desaturase to form LA, however, FAT-2 does not act on cVA [16,19,81]. The LA formed by FAT-2 is converted to ALA by FAT-1 Δ-15 desaturase or FAT-3, this is a step not found in mammals (Fig. 3) [19,21,22,86]. The Δ6 desaturation provided by FAT-3 is required for elongation of 18-carbon PUFAs to C20-

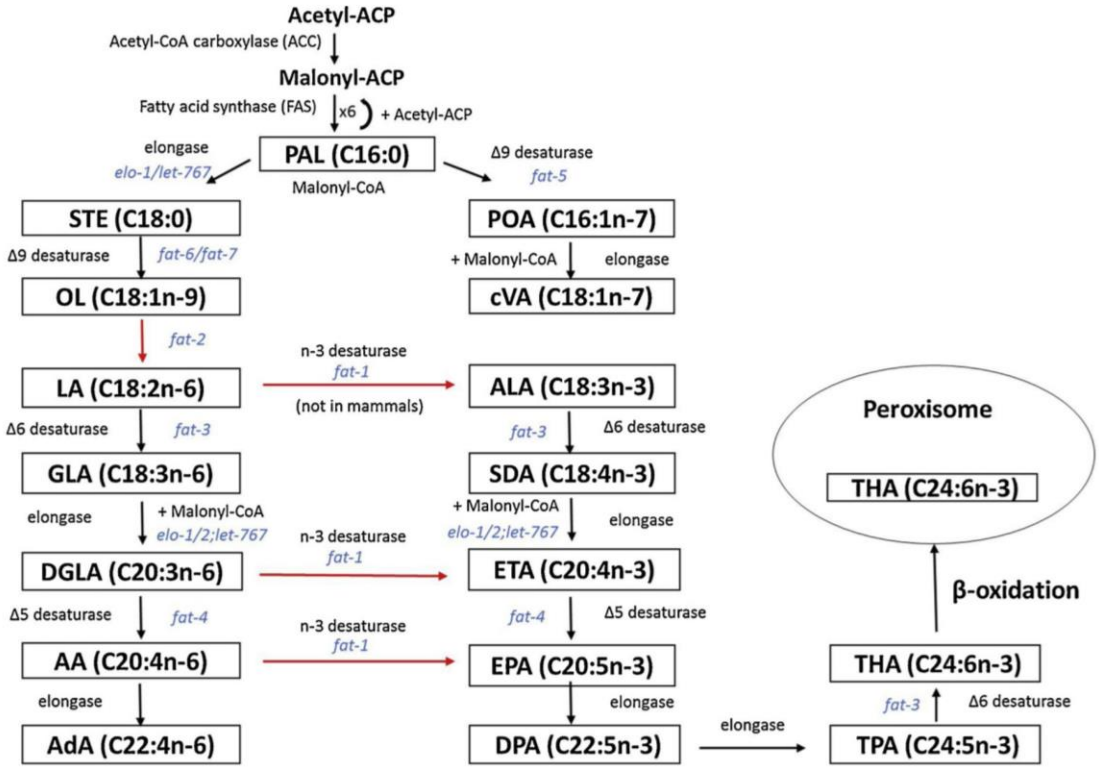


Fig. 3. A general polyunsaturated fatty acids biosynthesis pathway. Mammalian enzymes are drawn by solid black reaction arrows. Although this is a conserved pathway for both mammals and C. elegans, the nematode possess Δ12 and omega-3 desaturase enzymes that are solid red arrows. Interestingly, unlike mammals, C. elegans do not synthesize the 22 carbon PUFAs. Abbreviations: AA, arachidonic acid; ACC, acetyl-CoA carboxylase; ACP, acyl carrier proteins; Ada, docosatetraenoic acid; ALA, α-linolenic acid; cVA, cis-vaccenic acid; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; FAS, fatty acid synthase; GLA, γ-linolenic acid; LA, linolenic acid; OL, oleic acid; PAL, palmitic acid; POA, palmitoleic acid; SDA, stearidonic acid; STE, stearic acid; THA, tetracosahexaenoic acid (nisinic acid), TPA, tetracosapentaenoic acid.

PUFAs AA by ELO-1 and ELO-2 [21]. Both FAT-1 and FAT-4 modify the C20-PUFAs to produce EPA, respectively [21]. However, *C. elegans* does not possess a certain elongase activity required to form 22-carbon PUFAs (Watts; 2016). Therefore, the *C. elegans* fatty acid biosynthesis pathway stops at EPA, being the most predominant PUFA in *C. elegans* [21].

In *C. elegans*, 28 % of fatty acids in the total nematode lipids consists of C18 and C20 PUFAs [87]. Interestingly, PUFAs are not distributed evenly in the lipids of nematode, for instance, phosphatidylcholine consists of relatively high levels of PUFAs (56 % of total fatty acids), while triacylglycerol storage lipids contain relatively low levels of PUFAs (9 % of total fatty acids) [87]. The relative fatty acid composition of EPA is approximately 12.5 % followed by cVA with approximately 9.9 %, STE with about 5.2 % and LA with about 4.6 % of the total lipids [23]. However, AA levels are about 7-times lower (1.6 % of total lipids compared) to EPA levels [23]. Hillyard and German [32] demonstrated that wild-type nematodes grown at 20 °C produce significant levels of DGLA (0.097 ± 0.027 mg of fatty acids/g of nematode wet mass ± SEM), AA (0.043 ± 0.014 mg of fatty acids/g of nematode wet mass ± SEM) and EPA (0.511 ± 0.178 mg of fatty acids/g of nematode wet mass ± SEM) synthesized only in the presence of active Δ6 desaturase. However, due to loss of Δ6 desaturase activity, *fat-3(lg8101)* mutants had reduced levels of DGLA, AA and EPA, thus accumulating ALA and LIN, which are two Δ6 desaturase substrates [32]. Interestingly, *C. elegans* does not synthesize DHA, however it can be utilized [26,32]. Lesa and co-workers (2003) demonstrated that the supplementation of DHA to *fat-3* mutant culture restored locomotion in mutants to concentrations of wild-type. However, due to the retroconversion of DHA into EPA *in vivo*, the combination of both DHA and EPA rescued *fat-3* mutants fed DHA [26]. Moreover, Lesa and co-workers (2003) reported that both wild-type and *fat-3* mutant nematodes convert exogenously fed DHA to EPA. Thus speculated that DHA may be detrimental for *C. elegans* to possess in their membranes, therefore the nematodes use retroconversion of DHA to EPA mechanism to cope with DHA. In all *fat-3* mutant cultures supplemented with DHA, there was a significant increase in DHA concentrations relative to control. Interestingly supplementation of DHA or EPA in the *fat-3* mutants did not significantly have an effect on the life span of *fat-3* mutant. Moreover, *fat-3* mutant cultures supplemented with EPA and DHA revealed that DHA share a similar function to EPA in *C. elegans* [26]. In contrast to *C. elegans*, in mammals EPA can further be elongated to n-3 docosapentaenoic acid (DPA; 22:5n-3) which in turn can be converted via tetracosapentaenoic acid (TPA; 24:5n-3) to DHA [18,40,42]. These reactions occur at high rates, for example from EPA to DPA at 63 % and from DPA to DHA at 37 % [88]. The biosynthesis of EPA in mammals is synthesized from essential ALA, however, is found in low levels due to the rate limiting step of ALA desaturation to SDA in combination with consumption of high dietary LA and low ALA [10]. Therefore this in turn results in low levels of endogenous EPA in comparison to DHA. For instance, in wild-type mice on a standard sunflower oil based diet, EPA levels are < 0.05 % of total fatty acid in most tissues and blood [10]. Furthermore, metabolism by mammalian enzymes results in high relative levels of DHA concentrations (6.8 % of total fatty acid) compared to EPA (0.16 % of total fatty acid) in liver [10,89].

5. Oxylipins synthesis in *C. elegans*

The genome of *C. elegans* contains about 80 intact CYP450 genes that are assigned 2 to 16 independent families within the CYP superfamily, which could potentially produce hydroxy, epoxy and lipoxin products from DGLA [90]. However, researchers have been unable to identify any obvious mammalian orthologs of cyclooxygenases and lipoxygenases, or of prostanoid and leukotriene receptors in *C. elegans* genome [26,91]. Thus far, the enzymatic and biological functions of the individual CYP isoforms in *C. elegans* are still largely unknown. Most of the *C. elegans* closely related mammalian counterparts are members of the CYP2, CYP3, and CYP4 families [33,34,92]. In mammals, CYP2C and CYP2J enzymes catalyse the epoxidation, while CYP4A and CYP4F enzymes catalyse the hydroxylation of AA [33,34]. In *C. elegans*, eicosanoids are produced from PUFAs by CYP enzymes using two different pathways. One pathway uses hydroxylation to convert PUFAs, such as, AA to HETEs, while the other uses epoxygenase enzyme activity to produce epoxy-eicosatrienoic acids (EETs) [33]. In comparison to mammals, *C. elegans* produces AA and EPA-derived CYP eicosanoids mediated by CYP-33E2, CYP-29A3, and EMB-8, the nematode's NADPH-CYP reductase (CPR) enzymes [33,92]. For instance, CYP-33E2 prefers the substrate EPA rather than AA, and produces EPA-derived metabolite, 17, 18-epoxy-eicosatetraenoic (17, 18-EEQ) [34,92]. Interestingly, one of the major epoxygenase found in the mammalian heart, CYP2J2, has been shown to be a closely related CYP-33E2 human homolog. Moreover, it is presumed that CYP-29A3 contributes to the formation of hydroxy-metabolite and possesses a homologous sequence to CYP4 family members of mammals that forms the main AA-derived metabolite, 20-HETE [33]. Despite the early reports that *C. elegans* does not possess COX enzyme homologs responsible for synthesize of prostaglandins, recently using mass spectrometry (MS), they have identified several F-series prostaglandins, that includes prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and $PGF_{2\alpha}$ stereoisomers in lipid extracts of *C. elegans* [11,31,92]. These F-series prostaglandins are synthesized from several C20-PUFA precursors in the female germline and they function to attract sperm to oocytes (Fig. 4) [11,30,31]. In addition, a wide range of proteins with homology to mammalian phospholipases, PG synthases, PG reductases, PG transporters, and cytochrome P450 s are present in *C. elegans* genome [11]. Even though there is no precise mechanism of prostaglandin synthesize discovered, it is possible that *C. elegans* synthesize prostaglandins via COX-independent pathway [11,31,93–95]. Interestingly, these alternative COX-independent prostaglandin pathway is likely to be conserved in some mammalian species, due to the discovery of F-class prostaglandins in COX double knockout mice [19,94,96]. Altogether, the identification of *C. elegans* receptors for PUFAs and eicosanoids, as well as the identification of specific signal transduction pathways that regulate PUFA and eicosanoids, will allow for more mechanistic studies and understanding of lipid signalling in mammals.

6. PUFA function in *C. elegans*

6.1. Loss-of-function mutation

To understand the function of PUFAs, *C. elegans* has become a useful genetic model for evaluating the basic mechanisms that support the effects of specific PUFAs in a whole organism. This is important because despite n-6 and n-3 fatty PUFAs being structurally similar, their biological roles can be quite divergent. Over the past decades, the importance of PUFAs has been appreciated, since the discovery that n-3 and n-6 PUFAs are highly vital in the diet of mammals particularly for viability, thus termed essential fatty acids [36,37]. As mentioned earlier, the genome of *C. elegans* possess single genes that encodes Δ5, Δ6, Δ12 and n-3 desaturase enzymes, required to synthesis a series of n-3 and n-6 PUFAs. Moreover, there are three genes that particularly encodes Δ9 desaturases, necessary for producing MUFAs from SFA [21]. Mutant strains of *C. elegans* which lack these functional desaturase enzymes have been used to study the roles of specific fatty acids in longevity, reproduction and neurobiology [21,25,27,28]. Particularly, strains that carry mutations in the desaturase genes found in the early steps of the *C. elegans* PUFA biosynthesis pathway, for instance Δ6 (*fat-3* mutants), Δ9 (*fat-6* and *fat-7* double mutants) and Δ12 (*fat-2* mutants) desaturases have a more detrimental effect on the changes of lipid composition, and also severe phenotypic consequences, such as defects in growth, movement, and reproduction, compared to strains that carry mutations in desaturases functioning in the later steps of the pathway, such as Δ5 desaturase [21,24,25,27,28]. For instance, Δ12 desaturase *fat-2* mutants contain enormous amounts of OA while containing only 1

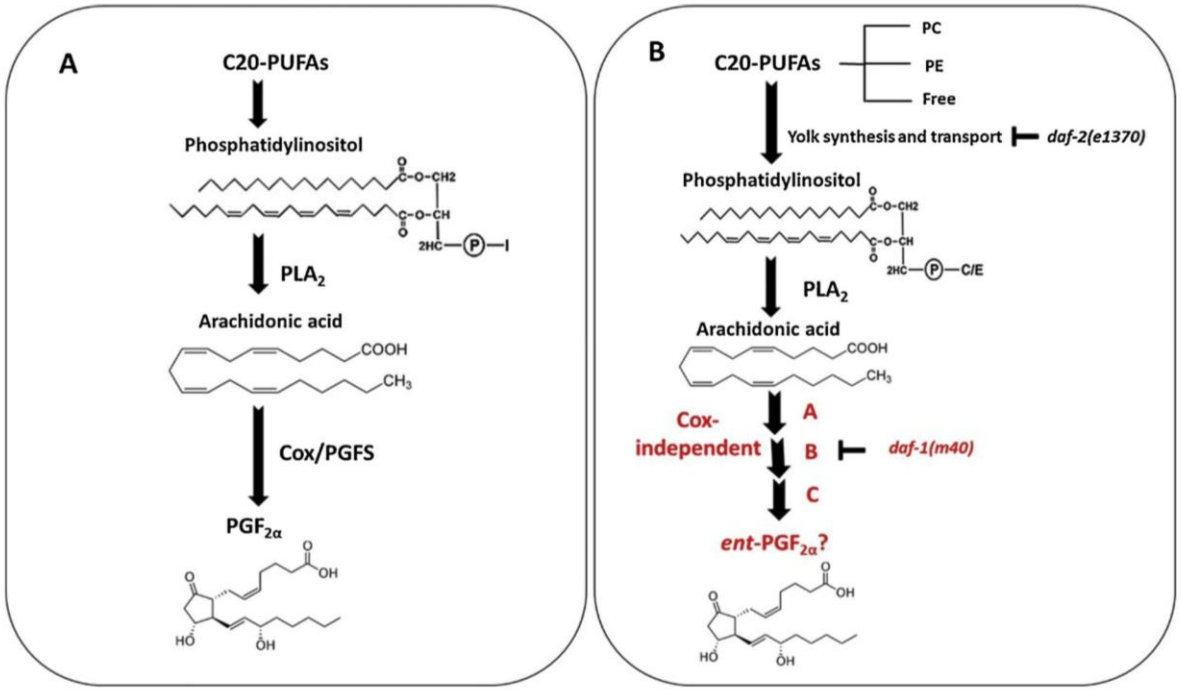


Fig. 4. Prostaglandin synthesis pathway in mammals and predicted *C. elegans* pathway. (A) In the COX pathway, phospholipase A_2 cleaves arachidonic acid (AA) from the sn-2 position of phospholipids like PI, generating lysoPI species lacking AA. Prostaglandins $F_{2\alpha}$ ($PGF_{2\alpha}$) is synthesized from free AA by the sequential actions of COX and prostaglandin F synthase (PGFS). (B) In *C. elegans*, C20-PUFAs are delivered to oocytes in yolk lipoprotein complexes. Yolk PUFAs are primarily found esterified to PC and PE, and in free forms. An incompletely understood metabolic Cox-independent pathway in oocytes, shown arbitrarily as steps A, B, and C, converts yolk lipids into F-series prostaglandins, such as ent-$PGF_{2\alpha}$ (or a co-eluting stereoisomer). Interestingly, *C. elegans* genome share similar enzyme homologs with mammals, which synthesize and modify PGs, however no PG G/H synthase is found in *C. elegans*. Abbreviations: C, choline; C20-PUFAs, 20 carbon polyunsaturated fatty acids; E, ethanolamine; I, inositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

% of PUFAs, resulting in many defects, including reduced brood size (~19 %), slow growth and uncoordinated movement, decrease hatching rate (29 %) and dramatically decreased lipid droplets content compared to wild-type nematodes [21,25,27,28]. While nematodes lacking a functional Δ6 desaturase *fat-3* contain carbon-18 PUFAs, however does not contain C20-PUFAs, thus are unable to synthesize any fatty acid beyond LA and ALA, and can cause C20-PUFA deficiency. Therefore leading to deficits in movement, slow growth, reduced brood size, abnormal body shape, defecation cycle, pharyngeal pumping activity, and basal innate immunity [21,25,27,26 demonstrated that the observed uncoordinated movement in *fat-3* mutants may be due to the depletion of synaptic vesicles and reduced release of neurotransmitter. Moreover, *fat-3* mutants are known to be defective in chemosensation, touch sensitivity, olfactory adaptation, and adaptation to alcohol [97–101] showed that the Δ9 desaturases *fat-6* and *fat-7* double mutants had a significant reduced turnover of fatty acyl groups, which function in optimal health span and longevity of the nematodes. Interestingly, although the *fat-4* and *fat-1* mutants contain diverse species of PUFAs and significantly diverse ratios of n-3 and n-6 species, their reproduction, growth, and movement are essentially normal [21].

The functions of n-3 and n-6 PUFAs in oocytes and embryo development of *C. elegans* are still in debate. According to previous studies it was not clear why the *fat-1* mutant strain showed no apparent defects in reproduction even though there was an increase in n-6 PUFAs levels compared to wild-type nematodes, and also non-detectable n-3 PUFAs, while *fat-2* and *fat-3* mutant strains showed reduced brood size and live progeny [21,25,27,97]. Recent studies revealed that the *fat-1* mutant has a high level of secreted yolk lipoprotein, however the oocyte lipid content is similar in both the *fat-1* mutant and wild-type [102]. More studies revealed that PUFA overexpression can negatively regulate DAF-16, promoting the yolk lipid protein expression level in the intestine [103], while some suggested that the n-6 PUFAs over-production in *fat-1* mutant results in the generation of plentiful yolk lipoprotein, exceeding beyond the uptake limit of oocytes [102]. Therefore, it was concluded that the *fat-1* mutant results in accumulation of abnormal yolk lipoprotein in pseudocoelom, however there was no apparent reproductive defect, thus suggesting that n-3 PUFAs do not play a critical role in the reproduction process [102]. Interestingly [93], showed that AA supplementation reduced yolk lipoprotein accumulation in pseudocoelom of *fat-2* transgenic nematodes [93]. While in 2003, Watts and co-workers reported that biochemical complementation with LA (18:3n-6) recovers the defect in reproduction observed in the *fat-3* mutant [25]. Together this findings provides a strong evidence that n-6 PUFAs, are required for transportation of yolk lipoprotein and development of oocyte in *C. elegans*, but not n-3 PUFAs [25,93]. Moreover, using the *Caenorhabditis elegans-Pseudomonas aeruginosa* host-pathogen model, it is demonstrated that Δ6 desaturase is required for basal innate immunity *in vivo*, through GLA and SDA, but not AA and EPA [104]. Furthermore, evidence supported that deficiencies in GLA and SDA result in increased susceptibility to *P. aeruginosa* bacterial infection, which is linked to reduced basal expression of numerous immune-specific genes encoding antimicrobial peptides [104]. Therefore, this renders GLA and SDA vital in maintaining basal activity of the p38 mitogen-activated protein kinase pathway, which functions in protecting most metazoan animals from various infections, and also from oxidative stress [104]. Recently, it was demonstrated that oleate is essential for the activation of innate immune response and resistance to bacterial infection in a distinctive manner compared to the effects of GLA and SDA. For instance, exogenous oleate was unable to rescue the enhanced susceptibility of the *fat-3(wa22)* mutant to pseudomonal infection [29]. Moreover, the *fat-6* and *fat-7* seemed to have an effect on pathogen susceptibility through oleate production, however

independent of PUFA biosynthesis via *fat-2* or *fat-3* enzymes [29].

6.2. Dietary supplementation

Interestingly, most of the above-mentioned deficiency can be rescued by dietary supplementation of the missing long-chain PUFAs to the mutant nematode strains [25,105–109]. According to Watts and Browse [105], dietary supplementation of the n-6 PUFA DGLA caused sterility, with DGLA concentration levels approaching approximately 12 % of total nematode fatty acids. These is mainly related to germ cell death due to apoptosis, as a result of the production of certain epoxy- and hydroxyl-toxic metabolites via CYP-33E2 activity [13,19]. Dietary supplementation of AA also resulted in germ cell death, however at a higher concentration than DGLA. On the contrary, supplementation with high amounts of n-3 EPA-rich fish oil consumption and n-6 LA, did not result in sterility, nor fertility, nor had a minor effect on germline development although supplemented at higher doses (greater than 35 % accumulation in nematode fatty acids) [105]. However, it resulted in a shorter lifespan due to oxidative stress resulting in accumulation of reactive lipid peroxidation products, thus causing damage to cell nucleic acids and proteins. Moreover, administration of fish oil containing EPA and DHA was able to enhance longevity, although too much intake of these fish oil shorten the lifespan of *C. elegans* [110]. Furthermore, Qi and co-workers (2017) demonstrated that ALA is capable of increasing the lifespan of *C. elegans* through NHR-49/PPARα activation and transcription factors of SKN-1/Nrf2. Another function of ALA is that it plays a physiologic role in the effects of germ line signalling on aging of nematodes [111]. These effects were observed through loss of germ line stem cells which resulted in an increase in fat synthesis with ALA as one of the fatty acids showing enhanced production [111].

Dietary supplementation of DGLA and AA in nematode culture media resulted in starvation resistance and also prolonged lifespan of nematodes [106–108]. Similarly, dietary supplementation with GLA can also increase nematode lifespan in a dose-dependent manner [112]. Furthermore, oxidized GLA generates oxylipins, including 9S-hydroperoxy-10E,12Z,15Z-octadecatrienoic acid which further results in an increase in longevity of GLA-treated nematodes [112]. Recently, Chen and co-workers (2019) demonstrated that apart from GLA and AA, other unsaturated fatty acids, such as OA, LA, and DGLA, excluding EPA, were able to restore the autophagy activities in *fat-7(wa36)* mutants. A similar observation was reported in wild-type *C. elegans* and mammalian cells, whereby dietary supplementation with DGLA and AA, but not with EPA, were able to activate autophagy [106,108]. Previously, two studies reported that at normal temperature, nematodes overexpressing *nhr-49* and the gain-of-function *nhr-49(et7)* mutants readily showed increased lifespan [113,114]. In addition [109], discovered that at low temperature, genetic inactivation of *fat-7*, *nhr-49* and *paqr-2* significantly reduced lifespan. However, at low temperature, supplementation of GLA and AA fully restored the lifespan in *fat-7(wa36)* mutant, but partially rescued the short lifespan in *nhr-49(nr2041)* and *paqr-2(tm3410)* mutants compared to wild-type nematodes [109]. Thus, at low temperature, the activation of the PAQR-2/NHR-49/FAT-7 signalling, which plays role in the adaptation to low temperature in larva, is involved in prolonged lifespan in adult nematodes [109]. Moreover, supplementation with MUFAs, OA, POA, or cVA is also sufficient to extend lifespan of *C. elegans* [106,108,115]. Fascinatedly, the inability of *C. elegans* mutant strains to synthesize C20-PUFAs can also result in impaired locomotion activity under normoxic conditions, and also where there is lack of the oxygen response [108]. However, long-term feeding with AA or EPA specifically rescued this impaired locomotion behaviour [108]. Moreover, C20-PUFAs also play a role in the touch sensation of C. elegans [100]. In contrast to impaired locomotion, only AA can rescue this phenotype [100]. Therefore it was concluded that C20-PUFAs modulate touch sensation whereas EPA- and AA-derived metabolites, rather than the parental C20-PUFAs themselves, regulate the locomotion activity of the C. elegans [100].

7. Oxylipin function in *C. elegans*

7.1. Guidance of sperm

Prostaglandins are known to be important for reproduction, however their regulatory mechanisms and functions are not well understood [3]. For instance, according to *in vitro* studies, prostaglandins can use the CatSper channel to induce Ca^{2+} influx into the sperm of a human [116,117]. But, the detailed biological function of this mechanism remains unclear, due to the difficulty of monitoring the behaviour of sperm in the reproductive tract of a female. However, to aid to the rescue, studies showed that *C. elegans* can be used to describe the mechanisms that function in guiding the sperm to maturing oocytes [30,31,93,105,118]. These authors discovered that *C. elegans* oocytes produce an important PUFA derived signal that recruits sperm for fertilization [30,31,93,105,118]. Follow up studies showed that these PUFAs, including AA and EPA, are synthesized in the intestine and transported to oocytes to be converted into F-series prostaglandins to guide the sperm under the regulation of the insulin signalling in the intestine and TGF-β pathway in sensory neurons, thus to completely control output of reproduction [30,31,93,94]. Fascinatedly, both signalling pathways responds when conditions are favourable such as during food availability. However during food scarcity, there is reduction in the biosynthesis of prostaglandin, thus leading to reduced effectiveness of sperm localizing the fertilization site, eventually decreasing the rate of fertilization. The mechanism for this regulation is as follows: in the intestine, there is a conversion of dietary fats to PUFAs, which are then incorporated into complexes of yolk lipoprotein (Fig. 5) [30]. Thereafter the yolk is secreted into the nematode pseudocoelom, were it flows straight to the gonad. As it reaches the gonad, it becomes endocytosed into oocytes by low-density lipoprotein receptor called receptor-mediated endocytosis 2 (RME-2) [119]. In the oocytes, the yolk provides C20-PUFAs that are in turn converted into about ten F-series prostaglandins that are structurally related and includes $PGF_{1\alpha}$ and $PGF_{2\alpha}$ stereoisomers [30,31]. These prostaglandins function collectively to promote the guidance of sperm to the site of fertilization, thus rendering the signalling of PUFAs important in oocytes for the recruitment of sperm to the spermatheca [31,120].

7.2. Pharyngeal pumping activity

As mentioned above, *C. elegans* CYP enzymes produce eicosanoids from PUFAs by way of two pathways. One pathway uses hydroxylation to convert PUFAs such as AA to HETEs, and another uses epoxygenase activity to generate EETs. [34] demonstrated that eicosanoid signalling can also function in pharyngeal cells, where it helps in the regulation of muscle contractions that are important for digestion. They also observed that one CYP isoform, CYP33E2, known to induce production of EET and HETE molecules in mammals, also functions in *C. elegans*. This CYP33E2 is one of the genes normally expressed in nematode pharynx (the organ responsible for rhythmically contraction to grind *E. coli* for digestion), however there can be a significant reduction in the pharyngeal contractions when the gene encoding CYP33E2 is deleted [34,121]. Moreover, this observed phenotype is shared by mutant nematodes of *fat-2* and *fat-3*, therefore supplying more evidence to the hypothesis that modulation of pharyngeal activity is via AA- and/or EPA-derived CYP eicosanoids [34,121]. Interestingly, majority of these impairments are mostly functional and not developmental, and can further be rescued by feeding AA and/or EPA to the mutant nematodes [21,26,104]. In addition, similarly, the short-term treatment with the most abundant *C. elegans* CYP eicosanoid, 17, 18-EEQ, was as effective in rescuing the nematode mutant strains as to long-term long-chain PUFA supplementation. In contrast, treatment of mutant nematodes with 20-HETE caused a decrease in pumping frequencies [121].

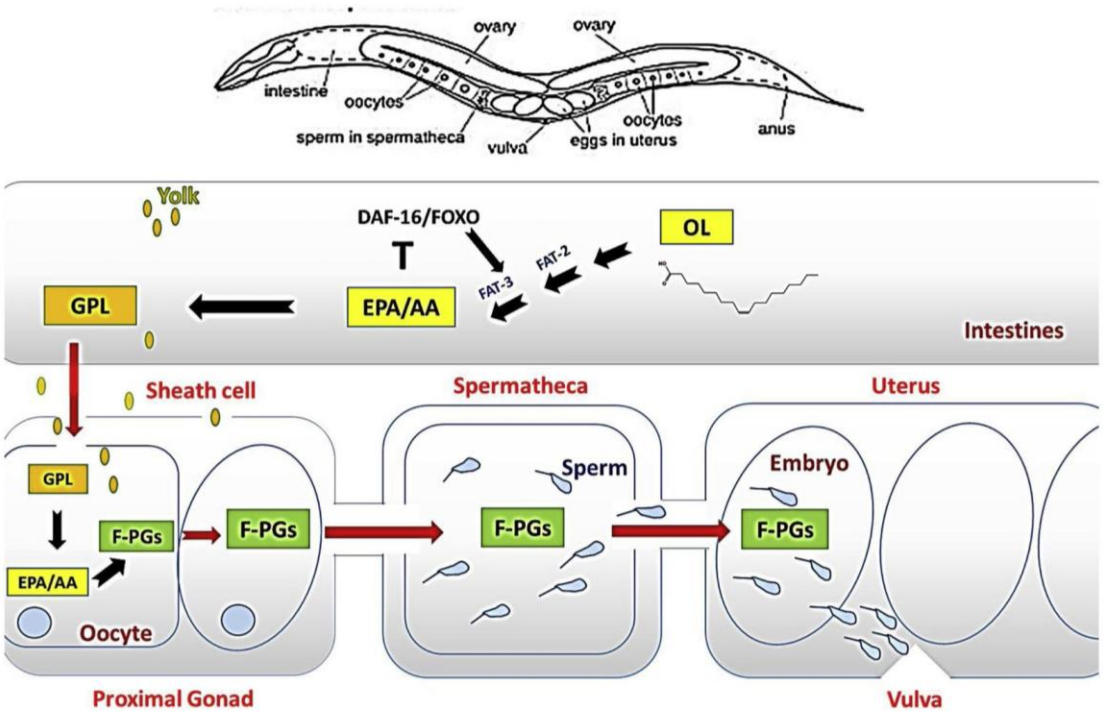


Fig. 5. Guidance of sperm to the in spermatheca for egg fertilization in *C. elegans* by eicosanoids, F-PGs. In the intestines, DAF-16/FOXO promotes biosynthesis of PUFA (AA or EPA) through increased fat-family genes expression. The PUFAs regulates DAF-16 negatively to promote oocyte yolk endocytosis and PG synthesis. Then GPL-containing yolk translocates to oocytes in order to synthesize F-PGs that facilitates in sperm guidance. Intercellular transportation of lipid molecules such as GPLs and F-PGs are indicated by red arrows. Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; F-PGs, F-series prostaglandins; GPL, glycerophospholipid; OL, oleic acid.

8. Conclusions and future research

The field of lipid biology in mammals is very much in its infancy, however one emerging theme discussed in this review is that lipid signalling seems to be vital in mammals, and often share functionally relevant lipid biosynthesis pathways with simple invertebrates. Thus, *C. elegans*, one of the most attractive and popular genetic model organism, is used to address questions and advance our understanding of the multiple physiological functions of PUFAs and oxylipins in several complex biological processes. For instance, due to simple physiology and genetic analysis of *C. elegans*, it has shown to be a useful model for studies of PUFA and oxylipin functions, particularly in development, reproduction, and longevity, since vertebrate models are more expensive, difficult and time consuming to manipulate [19]. Thus, as discussed in the review, studies performed in *C. elegans* regarding the functions of PUFAs and oxylipins clearly demonstrate their roles in slow growth, abnormal body shape, reduced brood size, movement, pharyngeal pumping activity, reproduction and defecation cycle. While it appears that many functions of C20 n-3 and n-6 PUFAs are redundant, many examples of specific functions for DGLA, AA and EPA are beginning to emerge [106,99,108,109,121]. For instance, AA is thought to be the most significant n-6 PUFA that is required as a constituent of membrane phospholipids, moreover it can act as a precursor for the biosynthesis of n-6 eicosanoids family [44,45]. Furthermore, it is observed that strains containing mutations in genes of the fatty acid desaturation pathway facilitate functional studies of PUFAs, since fatty acid composition can be manipulated both through dietary supplementation and genetic modifications [21,26,104,122,106,108,112]. Substantial evidence exists revealing the opposing effects of n-6 and n-3 PUFAs during the regulation of inflammation, with eicosanoids synthesized from n-6 PUFAs possessing pro-inflammatory effects, while those synthesized from n-3 PUFAs possess anti-inflammatory effects [14–17,2]. Interestingly, *C. elegans* have no clear mammalian COX enzyme homolog that synthesis prostaglandins, instead, it possess an alternative Cox-independent prostaglandin synthesis pathway that excludes the intermediates of prostaglandin-D or –E [31].

For future research, since thus far little is known about the effects of fatty acid-derived signals, more studies on lipid signalling in simple biological model systems will promote understanding of the regulation of lipid signalling pathways and their effects in biological processes. Moreover, improved metabolomic tools will assist in the discovery and identification of additional PUFA-derived signalling molecules in *C. elegans*. Thus far, studies on lipid signalling revealed that CYP eicosanoids play vital roles in the regulation of cardiac, renal and vascular function in mammals. Expectantly newly emerging fields may include the functions of CYP eicosanoids in the mammalian liver, lungs and gastrointestinal tract, however this investigations will be limited in simple invertebrate models like *C. elegans*, since it lack some of these essential mammalian organs. This limitation is arbitrary and partially reveals that the currently available knowledge on the biosynthesis and functions of CYP eicosanoid is relatively limited and further studies are still required. Recent research show that the CYP eicosanoid pathway is mostly susceptible to changes in composition of dietary fatty acid. Thus, a better understanding of the connection between CYP eicosanoids and PUFA may provide insight into the complex mechanisms that link dietary fatty acids to many biological processes. Thus, future studies promise to reveal important biological processes that depend on fatty acid signalling.

Acknowledgements

This work was supported by the National Research Foundation (NRF) of South Africa as part of the Research chair in Pathogenic Yeasts (Grant ID 115566) awarded to C Pohl. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

References

[1] G. van Meer, D.R. Voelker, G.W. Feigenson, Membrane lipids: where they are and how they behave, Nat. Rev. Mol. Cell Biol. 9 (2008) 112–124, https://doi.org/10.1038/nrm2330.

[2] A. Bouyanfif, S. Jayarathne, L. Koboziev, N. Moustaid-Moussa, The nematode *Caenorhabditis elegans* as a model organism to study metabolic effects of ω-3 polyunsaturated fatty acids in obesity, Adv. Nutr. 10 (2009) 165–178, https://doi.org/10.1093/advances/nmy059.

[3] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science 294 (2001) 1871–1875, https://doi.org/10.1126/science.294.5548.1871.

[4] A.A. Catala, Synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids, Biochem. Biophys. Res. Commun. 399 (2010) 318–323, https://doi.org/10.1016/j.bbrc.2010.07.087.

[5] M.J. Stables, D.W. Gilroy, Old and new generation lipid mediators in acute inflammation and resolution, Prog. Lipid Res. 50 (2011) 35–51, https://doi.org/10.1016/j.plipres.2010.07.005.

[6] K.A. Massey, A. Nicolaou, Lipidomics of polyunsaturated-fatty-acid-derived oxygenated metabolites, Biochem. Soc. Trans. 39 (2011) 1240–1246, https://doi.org/10.1042/BST0391240.

[7] T.L. Vrablik, J.L. Watts, Polyunsaturated fatty acid derived signaling in reproduction and development: insights from *Caenorhabditis elegans* and *Drosophila melanogaster*, Mol. Reprod. Dev. 80 (4) (2013) 244–259, https://doi.org/10.1002/mrd.22167.

[8] C. Arnold, A. Konkel, R. Fischer, W.H. Schunck, Cytochrome P450-dependent metabolism of omega-6 and omega-3 long-chain polyunsaturated fatty acids, Pharmacol. Rep. 62 (2010) 536–547, https://doi.org/10.1016/S1734-1140(10)70311-X.

[9] A.I. Ostermann, N.H. Schebb, Effects of omega-3 fatty acid supplementation on the pattern of oxylipins: a short review about the modulation of hydroxy-, dihydroxy-, and epoxy-fatty acids, Food Funct. 8 (2017) 2355, https://doi.org/10.1039/c7fo00403f.

[10] A.I. Ostermann, P. Waindok, M.J. Schmidt, C.-Y. Chiu, C. Smyl, N. Rohwer, et al., Modulation of the endogenous omega-3 fatty acid and oxylipin profile *in vivo*-A comparison of the *fat-1* transgenic mouse with C57BL/6 wildtype mice on an omega-3 fatty acid enriched diet, PLoS One 12 (9) (2017) e0184470, , https://doi.org/10.1371/journal.pone.0184470.

[11] E. Tiwary, M. Hu, M.A. Miller, J.K. Prasain, Signature profile of cyclooxygenase-independent F_2 series prostaglandins in *C. Elegans* and their role in sperm motility, Sci. Rep. 9 (2019) 11750, https://doi.org/10.1038/s41598-019-48062-y.

[12] J.K. Morris, D. Brian, B.D. Piccolo, C.S. John, Z.D. Green, J.P. Thyfault, S.H. Adams, Oxylipin profiling of Alzheimer's disease in nondiabetic and Type 2 diabetic elderly, Metabolites 9 (2019) 177, https://doi.org/10.3390/metabo9090177.

[13] M. Deline, J. Keller, M. Rothe, W.H. Schunck, R. Menzel, J.L. Watts, Epoxides derived from dietary dihomo-gamma-linolenic acid induce germ cell death in *C. Elegans*, Sci. Rep. 5 (2015) 15417, https://doi.org/10.1038/srep15417.

[14] D. Mozaffarian, J.H. Wu, Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events, J. Am. Coll. Cardiol. 58 (20) (2011) 2047–2067, https://doi.org/10.1016/j.jacc.2011.06.063.

[15] G. Zhang, D. Panigrahy, L.M. Mahakian, J. Yang, J.Y. Liu, K.S. Stephen Lee, H.I. Wettersten, A. Ulu, X. Hu, S. Tam, et al., Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 6530–6535, https://doi.org/10.1073/pnas.1304321110.

[16] W. Wang, J. Zhu, F. Lyu, D. Panigrahy, K.W. Ferrara, B. Hammock, G. Zhang, Omega-3 Polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer, Prostaglandins Other Lipid Mediat. 113–115 (2014) 13–20, https://doi.org/10.1016/j.prostaglandins.2014.07.002.

[17] P.C. Calder, Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance, Biochim. Biophys. Acta 1851 (4) (2015) 469–484, https://doi.org/10.1016/j.bbalip.2014.08.010.

[18] J.M. Lee, H. Lee, S. Kang, W.J. Park, Fatty acid desaturases, polyunsaturated fatty acid regulation, and biotechnological advances, Nutrient 8 (2016) 23, https://doi.org/10.3390/nu8010023.

[19] J.L. Watts, Using *Caenorhabditis elegans* to uncover conserved functions of omega-3 and omega-6 Fatty Acids, J. Clin. Med. 5 (2016) e19, https://doi.org/10.3390/jcm5020019.

[20] A.K. Corsi, B. Wightman, M. Chalfie, A transparent window into biology: a primer on *Caenorhabditis elegans*, Genetics 200 (2015) 387–407, https://doi.org/10.1534/genetics.115.176099.

[21] J.L. Watts, J. Browse, Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5854–5859, https://doi.org/10.1073/pnas.092064799.

[22] J.P. Spychalla, A.J. Kinney, J. Browse, Identification of an animal omega-3 fatty acid desaturase by heterologous expression in arabidopsis, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1142–1147, https://doi.org/10.1073/pnas.94.4.1142.

[23] J.L. Watts, M. Ristow, Lipid and carbohydrate metabolism in *Caenorhabditis elegans*, Genetics 207 (2) (2017) 413–446, https://doi.org/10.1534/genetics.117.300106.

[24] X. Jia, M. Xu, A. Yang, Y. Zhao, D. Liu, J. Huang, P. Proksch, W. Lin, Reducing effect of farnesylquinone on lipid mass in *C. Elegans* by modulating lipid metabolism, Mar. Drugs 17 (2019) 336, https://doi.org/10.3390/md17060336.

[25] J.L. Watts, E. Phillips, K.R. Griffing, J. Browse, Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *Caenorhabditis elegans fat-3* mutants, Genetics 163 (2003) 581–589.

[26] G.M. Lesa, M. Palfreyman, D.H. Hall, M.T. Clandinin, C. Rudolph, E.M. Jorgensen, G. Schiavo, Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. Elegans*, J. Cell. Sci. 116 (2003) 4965–4975, https://doi.org/10.1242/jcs.00918.

[27] T.J. Brock, J. Browse, J.L. Watts, Fatty acid desaturation and the regulation of adiposity in *Caenorhabditis elegans*, Genetics 176 (2007) 865–875, https://doi.org/10.1534/genetics.107.071860.

[28] Y.H. Yi, C.-H. Chien, W.-W. Chen, T.-H. Ma, K.-Y. Liu, Y.-S. Chang, T.-C. Chang, S.J. Lo, Lipid droplet pattern and nondroplet-like structure in two fat mutants of *Caenorhabditis elegans* revealed by coherentanti-Stokes Raman scattering microscopy, J. Biomed. Opt. 19 (2014) 011011, , https://doi.org/10.1117/1.JBO.19.1.01101.

[29] S.M. Anderson, H.K. Cheesman, N.D. Peterson, J.E. Salisbury, A.A. Soukas, R. Pukkila-Worley, The fatty acid oleate is required for innate immune activation and pathogen defense in *Caenorhabditis elegans*, PLoS Pathog. 15 (6) (2019), https://doi.org/10.1371/journal.ppat.1007893.

[30] H.M. Kubagawa, J.L. Watts, C. Corrigan, J.W. Edmonds, E. Sztul, J. Browse, M.A. Miller, Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment *in vivo*, Nat. Cell Biol. 8 (2006) 1143–1148, https://doi.org/10.1038/ncb1476.

[31] H.D. Hoang, J.K. Prasain, D. Dorand, M.A. Miller, A heterogeneous mixture of F-series prostaglandins promotes sperm guidance in the *Caenorhabditis elegans* reproductive tract, PLoS Genet. 9 (2013) 19, https://doi.org/10.1371/journal.pgen.1003271.

[32] S.L. Hillyard, J.B. German, Quantitative lipid analysis and life span of the *fat-3* mutant of *Caenorhabditis elegans*, J. Agric. Food Chem. 57 (2009) 3389–3396, https://doi.org/10.1021/jf8031414.

[33] J. Kulas, C. Schmidt, M. Rothe, W.H. Schunck, R. Menzel, Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*, Arch. Biochem. Biophys. 472 (2008) 65–75, https://doi.org/10.1016/j.abb.2008.02.002.

[34] M. Kosel, W. Wild, A. Bell, M. Rothe, C. Lindschau, C.E.W. Steinberg, W.H. Schunck, R. Menzel, Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of *Caenorhabditis elegans*, Biochem. J. 435 (2011) 689–700, https://doi.org/10.1042/BJ20101942.

[35] A.P. Simopoulos, Human requirement for n-3 polyunsaturated fatty acids, Poult. Sci. 79 (2000) 961–970, https://doi.org/10.1093/ps/79.7.961.

[36] G.O. Burr, M.M. Burr, On the nature and role of the fatty acids essential for nutrition, J. Biol. Chem. 86 (1930) 587–621, https://doi.org/10.1016/0163-7827(81)90004-7.

[37] J.G. Wallis, J.L. Watts, J. Browse, Polyunsaturated fatty acid synthesis: what will they think of next? Trends Biochem. Sci. 27 (2002) 467, https://doi.org/10.1016/S0968-0004(02)02168-0.

[38] W.S. Harris, D. Mozaffarian, E. Rimm, P. Kris-Etherton, L.L. Rudel, L.J. Appel, M.M. Engler, M.B. Engler, F. Sacks, Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention, Circulation 119 (6) (2009) 902–907, https://doi.org/10.1016/CIRCULATIONAHA.108.191627.

[39] A. Konkel, W.H. Schunck, Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids, Biochim. Biophys. Acta 1814 (2011) 210–222, https://doi.org/10.1016/j.bbapap.2010.09.009.

[40] G.C. Burdge, P.C. Calder, Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults, Reprod. Nutr. Dev. 45 (5) (2005), https://doi.org/10.1051/rnd:2005047 581 ± 597.

[41] L.M. Arterburn, E.B. Hall, H. Oken, Distribution, interconversion, and dose response of n-3 fatty acids in humans, Am. J. Clin. Nutr. 83 (2006) 1467S–1476S, https://doi.org/10.1093/ajcn/83.6.1467S.

[42] H. Guillou, D. Zadravec, P.G. Martin, A. Jacobsson, The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice, Prog. Lipid Res. 49 (2) (2010), https://doi.org/10.1016/j.plipres.2009.12.002 186 ± 199.

[43] R.J. Soberman, P. Christmas, The organization and consequences of eicosanoid signaling, J. Clin. Invest. 111 (2003) 1107–1113, https://doi.org/10.1172/JCI200318338.

[44] E.A. Dennis, J. Cao, Y.H. Hsu, V. Magrioti, G. Kokotos, Phospholipase A_2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention, Chem. Rev. 111 (2011) 6130–6185, https://doi.org/10.1021/cr200085w.

[45] I. Willenberg, A.I. Ostermann, N.H. Schebb, Targeted metabolomics of the arachidonic acid cascade: current state and challenges of LC-MS analysis of oxylipins, Anal. Bioanal. Chem. 407 (10) (2015) 2675–2683, https://doi.org/10.1007/s00216-014-8369-4.

[46] J.C. McGiff, J. Quilley, 20-HETE and the kidney: resolution of old problems and new beginnings, Am. J. Physiol. 277 (1999) R607–23, https://doi.org/10.1152/ajpregu.1999.277.3.R607.

[47] W.L. Smith, D.L. DeWitt, R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology, Annu. Rev. Biochem. 69 (2000) 145–182, https://doi.org/10.1146/annurev.biochem.69.1.145.

[48] D.L. Simmons, R.M. Botting, T. Hla, Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition, Pharmacol. Rev. 56 (2004) 387–437, https://doi.org/10.1124/pr.56.3.3.

[49] C.L. Bos, D.J. Richel, T. Ritsema, M.P. Peppelenbosch, H.H. Versteeg, Prostanoids and prostanoid receptors in signal transduction, Int. J. Biochem. Cell Biol. 36

(2004) 1187–1205, https://doi.org/10.1016/j.biocel.2003.08.006.
[50] H. Kuhn, J. Saam, S. Eibach, H.G. Holzhutter, I. Ivanov, M. Walther, Structural biology of mammalian lipoxygenases: enzymatic consequences of targeted alterations of the protein structure, Biochem. Biophys. Res. Commun. 338 (1) (2005) 93–101, https://doi.org/10.1016/j.bbrc.2005.08.238.
[51] M.W. Buczynski, D.S. Dumlao, E.A. Dennis, Thematic Review Series: proteomics. An integrated omics analysis of eicosanoid biology, J. Lipid Res. 50 (2009) 1015–1038, https://doi.org/10.1194/jlr.R900004-JLR200.
[52] Y.Y. Fan, R.S. Chapkin, Mouse peritoneal macrophage prostaglandin E_1 synthesis is altered by dietary gamma-linolenic acid, J. Nutr. 122 (1992) 1600–1606, https://doi.org/10.1093/jn/127.9.1765.
[53] O. Laneuville, D.K. Breuer, N. Xu, Z.H. Huang, D.A. Gage, J.T. Watson, M. Lagarde, D.L. DeWitt, W.L. Smith, Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and -2. Formation of 12-hydroxy-(9Z, 13E/Z, 15Z)-octadecatrienoic acids from alpha-linolenic acid, J. Biol. Chem. 270 (1995) 19330–19336, https://doi.org/10.1074/jbc.270.33.19330.
[54] C.N. Serhan, S. Hong, K. Gronert, S.P. Colgan, P.R. Devchand, G. Mirick, R.L. Moussignac, Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals, J. Exp. Med. 196 (2002) 1025–1037, https://doi.org/10.1084/jem.20020760.
[55] C.D. Buckley, D.W. Gilroy, C.N. Serhan, Proresolving lipid mediators and mechanisms in the resolution of acute inflammation, Immunity 40 (2014) 315–327, https://doi.org/10.1016/j.immuni.2014.02.009.
[56] J. Demarquoy, F. Le Borgne, Biosynthesis, metabolism and function of protectins and resolvins, Clin. Lipidol. 9 (6) (2014) 683–693, https://doi.org/10.2217/clp.14.44.
[57] H. Kuhn, S. Banthiya, K. van Leyen, Mammalian lipoxygenases and their biological relevance, Biochim. Biophys. Acta 1851 (2015) 308–330, https://doi.org/10.1016/j.bbalip.2014.10.002.
[58] A.A. Spector, H.Y. Kim, Cytochrome P epoxygenase pathway of polyunsaturated fatty acid metabolism, Biochim. Biophys. Acta 1851 (2015) 356–365, https://doi.org/10.1016/j.bbalip.2014.07.020.
[59] B. Samuelsson, S.E. Dahlen, J.A. Lindgren, C.A. Rouzer, C.N. Serhan, Leukotrienes and lipoxins: structures, biosynthesis, and biological effects, Science 237 (1987) 1171–1176, https://doi.org/10.1126/science.2820055.
[60] C.N. Serhan, J. Dalli, R.A. Colas, J.W. Winkler, N. Chiang, Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome, Biochim. Biophys. Acta 1851 (2015) 397–415, https://doi.org/10.1016/j.bbalip.2014.08.006.
[61] S. Feltenmark, N. Gautam, A. Brunnstrom, W. Griffiths, L. Backman, C. Edenius, L. Lindbom, M. Bjorkholm, H.E. Claesson, Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 680–685, https://doi.org/10.1073/pnas.0710127105.
[62] C.R. Pace-Asciak, Pathophysiology of the hepoxilins, Biochim. Biophys. Acta 1851 (2015) 383–396, https://doi.org/10.1016/j.bbalip.2014.09.007.
[63] R.J. Roman, P-450 metabolites of arachidonic acid in the control of cardiovascular function, Physiol. Rev. 82 (2002) 131–185, https://doi.org/10.1152/physrev.00021.2001.
[64] D. Wu, S.N. Meydani, n-3 polyunsaturated fatty acids and immune function, Proc. Nutr. Soc. 57 (1998) 503–509, https://doi.org/10.1079/PNS19980074.
[65] H.W. Harris, J.E. Gosnell, Z.L. Kumwenda, The lipemia of sepsis: triglyceride-rich lipoproteins as agents of innate immunity, J. Endotoxin Res. 6 (2000) 421–430, https://doi.org/10.1177/09680519000060060301.
[66] D. Swanson, R. Block, S.A. Mousa, Omega-3 fatty acids EPA and DHA: health benefits throughout life, Adv. Nutr. 3 (2012) 1–7, https://doi.org/10.3945/an.111.000893.
[67] E.F. Wiest, M.T. Walsh-Wilcox, M. Rothe, W.H. Schunck, M.K. Walker, Dietary omega-3 polyunsaturated fatty acids prevent vascular dysfunction and attenuate cytochrome P4501A1 expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin, Toxicol. Sci. 154 (2016) 43–54, https://doi.org/10.1093/toxsci/kfw145.
[68] J. Endo, M. Arita, Cardioprotective mechanism of omega-3 polyunsaturated fatty acids, J. Cardiol. 67 (2016) 22–27, https://doi.org/10.1016/j.jjcc.2015.08.002.
[69] X. Zhang, M.C. Rodriguez-Galan, J.J. Subleski, J.R. Ortaldo, D.L. Hodge, et al., Peroxisome proliferator-activated receptor-{gamma} and its ligands attenuate biologic functions of human natural killer cells, Blood 104 (2004) 3276–3284, https://doi.org/10.1182/blood-2004-02-0664.
[70] P. Gogolak, B. Rethi, I. Szatmari, A. Lanyi, B. Dezso, et al., Differentiation of CD1a2 and CD1a+ monocyte-derived dendritic cells is biased by lipid environment and PPAR {gamma}, Blood 109 (2007) 643–652, https://doi.org/10.1182/blood-2006-04-016840.
[71] M. Han, Twists and turns-how we stepped into and had fun in the "boring" lipid field, Sci. China Life Sci. 58 (2015) 1073–1083, https://doi.org/10.1007/s11427-015-4949-6.
[72] H. Zhang, N. Abraham, L.A. Khan, V. Gobel, RNAi-based biosynthetic pathway screens to identify *in vivo* functions of non-nucleic acid-based metabolites such as lipids, Nat. Protoc. 10 (2015) 681–700, https://doi.org/10.1038/nprot.2015.031.
[73] B.C. Mullaney, K. Ashrafi, *C. Elegans* fat storage and metabolic regulation, Biochim. Biophys. Acta 1791 (6) (2009) 474–478, https://doi.org/10.1016/j.bbalip.2008.12.013.
[74] K. Ashrafi, F.Y. Chang, J.L. Watts, A.G. Fraser, R.S. Kamath, et al., Genome wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes, Nature 421 (2003) 268–272, https://doi.org/10.1038/nature01279.
[75] C.T. Murphy, S.A. McCarroll, C.I. Bargmann, A. Fraser, R.S. Kamath, J. Ahringer, H. Li, C. Kenyon, Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*, Nature 424 (2003) 277–283, https://doi.org/10.1038/nature01789.
[76] K. Houthoofd, B.P. Braeckman, I. Lenaerts, K. Brys, A. De Vreese, S. Van Eygen, J.R. Vanfleteren, Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in *Caenorhabditis elegans*, Exp. Gerontol. 37 (2002) 1371–1378, https://doi.org/10.1016/S0531-5565(02)00173-0.
[77] C.L. Perez, M.R. Van Gilst, A 13C isotope labelling strategy reveals the influence of insulin signaling on lipogenesis in *C. Elegans*, Cell Metab. 8 (2008) 266–274, https://doi.org/10.1016/j.cmet.2008.08.007.
[78] K. Ashrafi, Obesity and the regulation of fat metabolism, WormBook (2007) 1–20, https://doi.org/10.1895/wormbook.1.130.1.
[79] M. Kniazeva, M. Sieber, S. McCauley, K. Zhang, J.L. Watts, M. Han, Suppression of the ELO-2 FA elongation activity results in alterations of the fatty acid composition and multiple physiological defects, including abnormal ultradian rhythms, in *Caenorhabditis elegans*, Genetics 163 (2003) 159–169.
[80] E.V. Entchev, D. Schwudke, V. Zagoriy, V. Matyash, A. Bogdanova, et al., LET-767 is required for the production of branched chain and long chain fatty acids in *Caenorhabditis elegans*, J. Biol. Chem. 283 (2008) 17550–17560, https://doi.org/10.1074/jbc.M800965200.
[81] X.R. Zhou, A.G. Green, S.P. Singh, *Caenorhabditis elegans* Δ12- desaturase FAT-2 is a bifunctional desaturase able to desaturate a diverse range of fatty acid substrates at the Δ12 and Δ15 positions, J. Biol. Chem. 286 (51) (2011) 43644–43650, https://doi.org/10.1074/jbc.M111.266114.
[82] M. Wang, H. Chen, Z. Gu, H. Zhang, W. Chen, Y.Q. Chen, ω-3 fatty acid desaturases from microorganisms: structure, function, evolution, and biotechnological use, Appl. Microbiol. Biotechnol. 97 (24) (2013) 10255–10262, https://doi.org/10.1007/s00253-013-5336-5.
[83] J.L. Watts, J. Browse, A palmitoyl-CoA-specific delta9 fatty acid desaturase from *Caenorhabditis elegans*, Biochem. Biophys. Res. Commun. 272 (2000) 263–269, https://doi.org/10.1006/bbrc.2000.2772.
[84] C.A. Rappleye, A. Tagawa, N. Le Bot, J. Ahringer, R.V. Aroian, Involvement of fatty acid pathways and cortical interaction of the pronuclear complex in *Caenorhabditis elegans* embryonic polarity, BMC Dev. Biol. 3 (2003) 8, https://doi.org/10.1186/1471-213X-3-8.
[85] T. Tanaka, K. Ikita, T. Ashida, Y. Motoyama, Y. Yamaguchi, K. Satouchi, Effects of growth temperature on the fatty acid composition of the free-living nematode *Caenorhabditis elegans*, Lipids 31 (1996) 1173–1178, https://doi.org/10.1007/BF02524292.
[86] M.M. Peyou-Ndi, J.L. Watts, J. Browse, Identification and characterization of an animal delta (12) fatty acid desaturase gene by heterologous expression in *Saccharomyces cerevisiae*, Arch. Biochem. Biophys. 376 (2000) 399–408, https://doi.org/10.1006/abbi.2000.1733.
[87] X. Shi, J. Li, X. Zou, J. Greggain, S.V. Rodkaer, N.J. Færgeman, B. Liang, J.L. Watts, Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase, J. Lipid Res. 54 (2013) 2504–2514, https://doi.org/10.1194/jlr.M039669.
[88] R.J. Pawlosky, J.R. Hibbeln, J.A. Novotny, N. Salem Jr, Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans, J. Lipid Res. 42 (8) (2001) 1257–1265.
[89] C. Schmocker, K.H. Weylandt, L. Kahlke, J. Wang, H. Lobeck, G. Tiegs, et al., Omega-3 fatty acids alleviate chemically induced acute hepatitis by suppression of cytokines, Hepatology 45 (4) (2007) 864–869, https://doi.org/10.1002/hep.21626.
[90] R. Menzel, T. Bogaert, R. Achazi, A systematic gene expression screen of *Caenorhabditis elegans* cytochrome P450 genes reveals CYP35 as strongly xenobiotic inducible, Arch. Biochem. Biophys. 395 (2001) 158–168, https://doi.org/10.1006/abbi.2001.2568.
[91] O. Gotoh, Divergent structures of *Caenorhabditis elegans* cytochrome P450 genes suggest the frequent loss and gain of introns during the evolution of nematodes, Mol. Biol. Evol. 15 (1998) 1447–1459, https://doi.org/10.1093/oxfordjournals.molbev.a025872.
[92] S.M. Lam, Z. Wang, J. Li, X. Huang, G. Shui, Sequestration of polyunsaturated fatty acids in membrane phospholipids of *Caenorhabditis elegans* dauer larva attenuates eicosanoid biosynthesis for prolonged survival, Redox Biol. 12 (2017) 967–977, https://doi.org/10.1016/j.redox.2017.05.002.
[93] J.W. Edmonds, J.K. Prasain, D. Dorand, Y. Yang, H.D. Hoang, J. Vibbert, H.M. Kubagawa, M.A. Miller, Insulin/FOXO signaling regulates ovarian prostaglandins critical for reproduction, Dev. Cell 19 (2010) 858–871, https://doi.org/10.1016/j.devcel.2010.11.005.
[94] K. McKnight, H.D. Hoang, J.K. Prasain, N. Brown, J. Vibbert, et al., Neurosensory perception of environmental cues modulates sperm motility critical for fertilization, Science 344 (2014) 754–757, https://doi.org/10.1126/science.1250598.
[95] J.K. Prasain, L. Wilson, H.D. Hoang, R. Moore, M.A. Miller, Comparative lipidomics of *Caenorhabditis elegans* metabolic disease models by swath non-targeted tandem mass spectrometry, Metabolites 5 (2015) 677–696, https://doi.org/10.3390/metabo5040677.
[96] B. Pier, J.W. Edmonds, L. Wilson, A. Arabshahi, R. Moore, G.W. Bates, J.K. Prasain, M.A. Miller, Comprehensive profiling of prostaglandins in human ovarian follicular fluid using mass spectrometry, Prostaglandins Other Lipid Mediat. 134 (2018) 7–15, https://doi.org/10.1016/prostaglandins.2017.11.001.
[97] A.H. Kahn-Kirby, J.L. Dantzker, A.J. Apicella, W.R. Schafer, J. Browse, et al., Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling *in vivo*, Cell 119 (2004) 889–900, https://doi.org/10.1016/j.cell.2004.11.005.
[98] D.M. O'Halloran, S. Altshuler-Keylin, J.I. Lee, N.D. L'Etoile, Regulators of AWC-mediated olfactory plasticity in *Caenorhabditis elegans*, PLoS Genet. 5 (2009) e1000761, , https://doi.org/10.1371/journal.pgen.1000761.

[99] R.C. Raabe, L.D. Mathies, A.G. Davies, J.C. Bettinger, The omega-3 fatty acid eicosapentaenoic acid is required for normal alcohol response behaviors in *C. Elegans*, PLoS One 9 (2014) e105999, , https://doi.org/10.1371/journal.pone.0105999.

[100] V. Vasquez, M. Krieg, D. Lockhead, M.B. Goodman, Phospholipids that contain polyunsaturated fatty acids enhance neuronal cell mechanics and touch sensation, Cell Rep. 6 (2014) 70–80, https://doi.org/10.1016/j.celrep.2013.12.012.

[101] B.C. Dancy, S.W. Chen, R. Drechsler, P.R. Gafken, C.P. Olsen, 13C- and 15N-Labeling strategies combined with mass spectrometry comprehensively quantify phospholipid dynamics in *C. Elegans*, PLoS One 10 (2015) e0141850, , https://doi.org/10.1371/journal.pone.0141850.

[102] W.W. Chen, Y.H. Yi, C.H. Chien, K.C. Hsiung, T.H. Ma, Y.C. Lin, S.J. Lo, T.C. Chang, Specific polyunsaturated fatty acids modulate lipid delivery and oocyte development in*C. elegans* revealed by molecular-selective label free imaging, Sci. Rep. 6 (2016) 32021, https://doi.org/10.1038/srep32021.

[103] S. Kim, C. Spike, D. Greenstein, Control of oocyte growth and meiotic maturation in *Caenorhabditis elegans*, in: T. Schedl (Ed.), In Germ Cell Development in *C. Elegans*, Springer, New York, NY, 2013, pp. 277–320.

[104] M. Nandakumar, M.W. Tan, Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity, PLoS Genet. 4 (2008) e1000273, , https://doi.org/10.1371/journal.pgen.1000273.

[105] J.L. Watts, J. Browse, Dietary manipulation implicates lipid signaling in the regulation of germ cell maintenance in *C. elegans*, Dev. Biol. (Basel) 292 (2006) 381–392, https://doi.org/10.1016/j.ydbio.2006.01.013.

[106] E.J. O'Rourke, P. Kuballa, R. Xavier, G. Ruvkun, Omega-6 polyunsaturated fatty acids extend life span through the activation of autophagy, Genes Dev. 27 (2013) 429–440, https://doi.org/10.1101/gad.205294.112.

[107] D. Navarro-Herrera, P. Aranaz, L. Eder-Azanza, M. Zabala, C. Hurtado, A. Romo-Hualde, J.A. Martínez, C.J. González-Navarro, J.L. Vizmanos, Dihomo-gamma-linolenic acid induces fat loss in *C. elegans* in an omega-3-independent manner by promoting peroxisomal fatty acid β-oxidation, Food Funct. 9 (2018) 1621, https://doi.org/10.1039/c7fo01625e.

[108] K. Papsdorf, A. Brunet, Linking lipid metabolism to chromatin regulation in aging, Trends Cell Biol. 1461 (2018) 1–20, https://doi.org/10.1016/j.tcb.2018.09.004.

[109] Y.-L. Chen, J. Tao, P.-J. Pei-Ji Zhao, W. Tang, J.-P. Xu, K.-Q. Zhang, C.-G. Zou, Adiponectin receptor PAQR-2 signaling senses low temperature to promote *C. elegans* longevity by regulating autophagy, Nat Com 10 (2019) 2602, https://doi.org/10.1038/s41467-019-10475-8.

[110] S. Sugawara, T. Honma, J. Ito, R. Kijima, T. Tsuduki, Fish oil changes the lifespan of *Caenorhabditis elegans* via lipid peroxidation, J. Clin. Biochem. Nutr. 52 (2013) 139–145, https://doi.org/10.3164/jcbn.12-88.

[111] F.R. Amrit, E.M. Steenkiste, R. Ratnappan, S.W. Chen, T.B. McClendon, D. Kostka, J. Yanowitz, C.P. Olsen, A. Ghazi, DAF-16 and TCER-1 facilitate adaptation to germline loss by restoring lipid homeostasis and repressing reproductive physiology in *C. elegans*, PLoS Genet. 12 (2016) e1005788, , https://doi.org/10.1371/journal.pgen.1005788.

[112] W. Qi, G.E. Gutierrez, X. Gao, H. Dixon, J.A. Joe A. McDonough, A.M. Marini, A.L. Fisher, The ω-3 fatty acid α-linolenic acid extends *Caenorhabditis elegans* lifespan via NHR-49/PPARα and oxidation to oxylipins, Aging Cell 16 (2017) 1125–1135, https://doi.org/10.1111/acel.12651.

[113] R. Ratnappan, F.R.G. Amrit, S.-W. Chen, H. Gill, K. Holden, J. Ward, et al., Germline signals deploy NHR-49 to modulate fatty-acid beta-oxidation and desaturation in somatic tissues of *C. elegans*, PLoS Genet. 10 (2014) e1004829, , https://doi.org/10.1371/journal.pgen.100829.

[114] K. Lee, G.Y. Goh, M.A. Wong, T.L. Klassen, S. Taubert, Gain-of function alleles in *Caenorhabditis elegans* nuclear hormone receptor *nhr-49* are functionally distinct, PLoS One 11 (2016) e0162708, , https://doi.org/10.1371/journal.pone.0162708.

[115] S. Han, E.A. Schroeder, C.G. Silva-Garcia, K. Hebestreit, W.B. Mair, et al., Monounsaturated fatty acids link H3K4me3 modifiers to *C. elegans* lifespan, Nature 544 (2017) 185–190, https://doi.org/10.1038/nature21686.

[116] T. Strünker, N. Goodwin, C. Brenker, N.D. Kashikar, I. Weyand, R. Seifert, U.B. Kaupp, The CatSper channel mediates progesterone-induced Ca^{2+} influx in human sperm, Nature 471 (2011) 382–386, https://doi.org/10.1038/nature09769.

[117] P.V. Lishko, I.L. Botchkina, Y. Kirichok, Progesterone activates the principal Ca^{2+} channel of human sperm, Nature 471 (2011) 387–391, https://doi.org/10.1038/nature09767.

[118] J.W. Edmonds, S.L. McKinney, J.K. Prasain, M.A. Miller, The gap junctional protein INX-14 functions in oocyte precursors to promote *C. elegans* sperm guidance, Dev. Biol. 359 (2011) 47–58, https://doi.org/10.1016/j.ydbio.2011.08.014.

[119] B. Grant, D. Hirsh, Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte, Mol. Biol. Cell 10 (1999) 4311–4326, https://doi.org/10.1091/mbc.10.12.4311.

[120] J.L. Watts, Fat synthesis and adiposity regulation in *Caenorhabditis elegans*, Trends Endocrinol. Metab. 20 (2009) 58–65, https://doi.org/10.1016/j.tem.2008.11.002.

[121] Y. Zhou, J.R. Falck, M. Rothe, W.H. Schunck, R. Menzel, Role of CYP eicosanoids in the regulation of pharyngeal pumping and food uptake in *Caenorhabditis elegans*, J. Lipid Res. 56 (2015) 2110–2123, https://doi.org/10.1194/jlr.M061887.

[122] M.L. Deline, T.L. Vrablik, J.L. Watts, Dietary supplementation of polyunsaturated fatty acids in *Caenorhabditis elegans*, J. Vis. Exp. 81 (2013) 50879, https://doi.org/10.3791/50879.

3. Section C

Section C entails additional information to complete the literature review for this dissertation. This section is thereafter followed by general conclusions of chapter 1 and the aim and objectives of the dissertation.

3.1. Effects of infection on fat metabolism

Studies using clinical and animal models have become very crucial in understanding the mechanisms mediating the effects of PUFAs and oxylipins. However, these studies are time consuming, expensive, and often limited for research studies, due to ethical concerns. Therefore, there is an urgent need for affordable and efficient animal models for scaled screening of various bioactive compounds or pharmacologic agents in various diseases. In this review, we mainly focused on *C*. *elegans* as an affordable, convenient, and metabolically relevant model organism to understanding the mechanisms that mediate the roles of PUFAs and oxylipins on infections and host immunity. *C. elegans* can easily be infected by various human pathogens and readily open to different molecular tools, thus an important host model in order to understand the diverse facets of host-pathogen interaction including innate immunity pathways (Aballay and Ausubel, 2002). These traits of the nematode, joined together with a high degree of conservation with human innate immune signaling pathways, makes *C. elegans* crucial for drug discovery studies (Artal-Sanz *et al*., 2006; Burns *et al*., 2006). The co-existence of both host and pathogen in a host-pathogen interaction assists in the identification of chemical entities able to rescue infected hosts. Interestingly, this may result in the discovery of molecules that leads to the attenuation of bacterial virulence or augment the host immunity (Moy *et al*., 2006). Over the years, the usage of *C. elegans* in host-pathogen screening assays have expanded to many human pathogens, namely *Enterococcus faecalis* (Moy *et al*., 2006), *C. albicans* (Breger *et al*., 2007), *Vibrio alginolyticus* (Durai *et al*., 2013), *Staphylococcus aureus* (Kong *et al*., 2014), *Burkholderia pseudomallei* (Eng and Nathan, 2015), and *Salmonella enteritidis* (Kulshreshtha *et al*., 2016). Majority of the known Gram-positive and Gram-negative bacteria as well as yeast pathogens that infect *C. elegans* make use of two main routes of infection. They can either contact the nematode at the intestinal epithelium surfaces establish an intestinal infection after being ingested through the pharynx during feeding or as bacteria or fungi adhere to the nematode cuticle and epidermis (Powell and Ausubel, 2008). Examples of the latter include two Gram-negative species, *Yersinia* spp. and *Xenorhabdus nematophilum*, able to form a biofilm on the head of the nematode (Darby *et al*., 2002), the Gram-positive bacterium *Microbacterium nematophilum*,

that can adhere to a specific region on the tail (Hodgkin *et al*., 2000), and fungus *Drechmeria coniospora*, with spores that attaches to the nematode cuticle thereafter penetrate the cuticle and epidermis (Dijksterhuis *et al*., 1990). Interestingly, as *D. coniospora* establishes an infection in *C. elegans*, it provokes the rapid induction of genes that encodes antimicrobial peptides (AMPs), including genes found in the *nlp* family (Couillault *et al*., 2004; Pujol *et al*., 2008). Moreover, damage of the epidermis, triggers *C. elegans* to expresses AMPs, such as *nlp-29* that can be involved in a wound healing response, which in parallel involves cellular repair (Pujol *et al*., 2008). After injury or infection, the expression of *nlp-29* depends on a PKC-p38 MAPK pathway that can act in the epidermis as cell autonomous (Ziegler *et al*., 2009).

Since *C. elegans* is a bacterivore, most of the intestinal infections are easily achieved through feeding, thus resulting in the nematode relatively ideal for investigating interactions with gut bacteria (Corsi *et al*., 2015). Sim and Hibberd (2016) identified potentially pathogenic host-microbiome interactions, and thereafter made a selection of nematode candidate genes whose orthologues in the human host have previously been involved in metabolic or inflammatory diseases. The FA metabolism and epithelial junction integrity which are involved in *C. elegans* defence against *E. faecalis*, which is commonly associated with hospital-acquired, multidrug-resistant infections were the two aspects of nematode biology that were identified in the RNA interference (RNAi) screen of these genes (Palmer *et al*., 2010). An RNAi screen of 17 nematode candidate genes showed that the knockdown of the master regulator of fat metabolism, *nhr-49* transcription factor, can shorten the lifespan of the nematode infected with *E. faecalis* in comparison to its food source, *Escherichia coli* (Palmer *et al*., 2010). Notably, the effects of *nhr-49*'s on fat metabolism and lifespan are more closely analogous to the mammalian peroxisome proliferator-activated receptors (PPARs), which are nuclear hormone receptor transcription factors activated by FAs and their derivatives, and also known as key regulators of metabolism and controllers of inflammation (Gilst *et al*., 2005; Wahli and Michalik, 2012). Therefore, the functional similarity between *nhr-49* and the mammalian PPARs proposes that this is mediated via the association between FA metabolism and innate immunity (Gilst *et al*., 2005; Palmer *et al*., 2010; Wahli and Michalik, 2012). More studies were performed by Dasgupta and co-workers (2020) that investigated the effect of lipid metabolism regulation on immune response and impact on nematode survival after pathogenic bacterial infection. The authors discovered that a diet with either *E. faecalis* or *S. aureus* may lead to a rapid utilization of lipid droplets (LDs) stores in the intestines of *C. elegans* (Dasgupta *et al*., 2020). Moreover, the diet of *E. faecalis* can result developmental arrest in nematode larvae and in growth arrest in adult nematodes. Further studies demonstrated that the diet of *E. faecalis* have the ability to activate the whole program of neutral lipid hydrolysis, while the neutral lipid synthesis is suppressed (Dasgupta *et al*., 2020).

The FAs Beta-oxidation and the production of the immune effector are dependent on a nuclear hormone receptor and PPARα orthologue, NHR-49 (Dasgupta *et al*., 2020). An increase in LDs levels boosts *C. elegans* survival on *E. faecalis* in a manner that is NHR-49 dependent. Thus, Dasgupta and co-workers (2020) showed that *C. elegans* survival from *E. faecalis* infection needs NHR-49 dependent immunometabolic axis activation.

3.2. The interplay between PUFAs and innate immunity in *C. elegans*

As mentioned above, in nature, *C. elegans* is found as a free-living soil nematode known to feed on bacteria as a food source, and constantly encounter several threats from ingested pathogens (Irazoqui *et al*., 2010). Using *C. elegans* as a model host, numerous studies have provided genetic and genomic tools in the field of host-pathogen interactions pertaining to the nematode defence mechanisms against different bacterial, fungal and microsporidial pathogens. Similar to other invertebrate species, this nematode lacks an adaptive immune system (Pukkila-Worley *et al*., 2011). However, contrary to majority of the invertebrates, *C. elegans* does not possess specialized immune cells (Cohen and Troemel, 2015). Thus, the nematode have three major defence mechanisms against attacks by microbial pathogens, as briefly described below (Barrière and Felix, 2006).

The first line of defence entails avoidance behaviour that relies on chemosensory neurons that sense pathogens and induce escape (Wes and Bargmann, 2001; Pujol *et al*., 2001; Pradel *et al*., 2007). This is triggered by recognising the pathogen through Toll-like receptor, TOL-1, and detecting microbial molecules like cyclic pentadepsipeptide biosurfactant, serrawettin W2 (Wes and Bargmann, 2001; Pujol *et al*., 2001; Pradel *et al*., 2007; Peng *et al*., 2018). It has been shown that nematodes have the ability to differentiate between different pathogens (Shtonda and Avery, 2006). Although some of the pathogens seem to attract *C. elegans*, others are known to repel this nematode thus resulting in avoidance behaviour (Peng *et al*., 2018).

The second axis of protection that *C. elegans* uses against pathogen invasion is through physical barriers such as a strong cuticle and pharyngeal grinder (Labrousse *et al*., 2000; Kim *et al*., 2002; Schulenburg *et al*., 2004). The strong cuticle of *C. elegans* is made of chitin and, collagen, and can act as a physical barrier resistant to puncturing (Chisholm and Hsiao 2012; Sandhu *et al*., 2021). As a complement, the nematode pharyngeal grinder functions to destroy pathogens present during feeding by preventing them from moving to the intestine and causing infection.

The third line of defence includes inducible mechanisms whereby, if the pathogen cannot be avoided, the nematode triggers specific innate immunity mechanisms, then provokes expression of antimicrobial polypeptides (Irazoqui *et al*., 2010). Coordination of these defences includes a number of highly conserved elements possessing mammalian orthologues (Kim *et al*., 2002; Pukkila-Worley *et al*., 2011). However, unlike humans, the nematode relies solely on innate immune defence since it lacks an adaptive immune system (Pukkila-Worley *et al*., 2011). Intriguingly, there is a striking resemblance between the *C. elegans* intestinal epithelial cells and human intestinal cells, however, due to the nematodes lacking of both circulatory system and cells involved in immune response, their intestinal epithelium establishes the primary line of defence against ingested pathogens (Troemel *et al*., 2008). Thus, possible to conduct appropriate analyses for mechanisms underlying innate immune in this *C. elegans* model, which will be the main focus of this chapter.

Innate immunity is an ancient and conserved system that serves as the first line of defence against invading pathogens. The innate immune system can be differentiated as either constitutive or basal branch of innate immune defence or as the pathogen-induced responses, however both have overlapping components (Schmid-Hempel and Ebert, 2003; Schulenburg *et al*., 2004). Constitutive or basal immunity entails a constant production of effector molecules including defensins and other AMPs, that provides a barrier of prevention and allow the organism to respond instantaneously towards any immunological insult (Zhao *et al*., 1996; O'Neil *et al*., 1999; Ooi *et al*., 2002). The inducible branch of the innate immune system, on the other hand, is activated after the host has been in contact with the pathogen (Lemaitre *et al*., 1997). This innate immune system involves the induction of additional effector molecules, and the phagocytic cells recruitment and activation where needed (Lemaitre *et al*., 1997). Interestingly, both the constitutive and inducible innate immunity have been intensively described in *C. elegans*. For instance, numerous AMPs, such as lysozymes, the ABF-2 defensin and the SPP-1 saposin are constitutively expressed in healthy nematodes (Kato *et al*., 2002; Alper *et al*., 2007). Research revealed that after infection, a subset of these constitutively-expressed AMPs, and a set of other effector molecules, including the C-type lectin family, can be up-regulated at various time points (Mallo *et al*., 2002; Shapira *et al*., 2006; Troemel *et al*., 2006). Some AMPs and proteins, such as cytokines function as effector mechanisms of innate immune response to control infection (Figure 1.1).

Secondly, when *C. elegans* is infected, it uses receptors to recognize a pathogen or damages induced by a pathogen (Peng *et al*., 2018). *Caenorhabditis elegans* Toll-like receptors (TLRs) function by launching an applicable immune response against a pathogen (Pujol *et al*., 2001; Brandt and Ringstad, 2015; Peng *et al*., 2018). The TLRs share a similar structure, and contain

an intracellular Toll-Interleukin-1 receptor (TIR) domain and ectodomain of leucine-rich repeats (Botos *et al*., 2011). A TIR domain adaptor protein TIR-1, orthologous to mammalian sterile alpha and TIR motif-containing protein 1 (SARM), usually functions by activating the PMK-1 pathway in *C. elegans* innate immunity (Liberati *et al*., 2004). Thirdly, a complex innate immune response is triggered, involving signalling cascades that use proteins and transcriptional regulators to direct changes in gene expression (Figure 1.1) (Kim *et al*., 2002; Kurz and Ewbank, 2003; Schulenburg *et al*., 2004; Irazoqui *et al*., 2010). The three well characterized core signal transduction pathways are DBL-1 pathway, mitogen-activated protein kinase (MAPK) pathway (Kim *et al*., 2002) and DAF-2/DAF-16 pathway, an insulin-like defence pathway that results in the activation of the FOXO transcription factor homologue DAF-16 (Garsin *et al*., 2003; Murphy *et al*., 2003). These pathways are important for the increased production of numerous effector molecules, such as lectins, lysozymes and AMPs, to levels higher than those observed under basal conditions, found in healthy nematodes (Troemel *et al*., 2006; Alper *et al*., 2007). For instance, MAPK pathway has a central role in resistance to microbial pathogens. Furthermore, regulators of MAPK, such as p38 MAP kinase homologue PMK-1, have been seen to have an essential role in MAPK pathway, which regulates innate immunity in *C. elegans*. Interestingly, there has been many implicating studies about MAPK pathway pertaining host resistance to majority of fungal and bacterial pathogens (Kim *et al*., 2002). The p38 MAP kinase pathway is also essential in conserving the basal immune response, and mutant strains disrupted for key components of this pathway, including p38 MAP kinase mutant *pmk-1*, possess defects in the constitutive expression of lectins, lysozymes and other effector molecules (Troemel *et al*., 2006).

Pathogens such as *C. albicans*, with cell walls that consist of chitin polysaccharides, mannan, and glucan can be easily recognised by the host (Gow and Hube, 2012; Gow *et al*., 2012). Since *C. albicans* yeast form can result in an intestinal infection in *C. elegans*, the host response towards this pathogen is mostly through the mediation of pattern recognition, pathogen-associated molecular patterns (PAMPs). According to Pukkila-Worley *et al*. (2011), during response of *C. elegans* to fungal pathogens the transcription of antibacterial immune effectors of nematodes are selectively repressed. Thus suggesting that *C. elegans* can take advantage by irrespectively mounting particular antifungal defences at the cost of antibacterial responses (Pukkila-Worley *et al*., 2011).

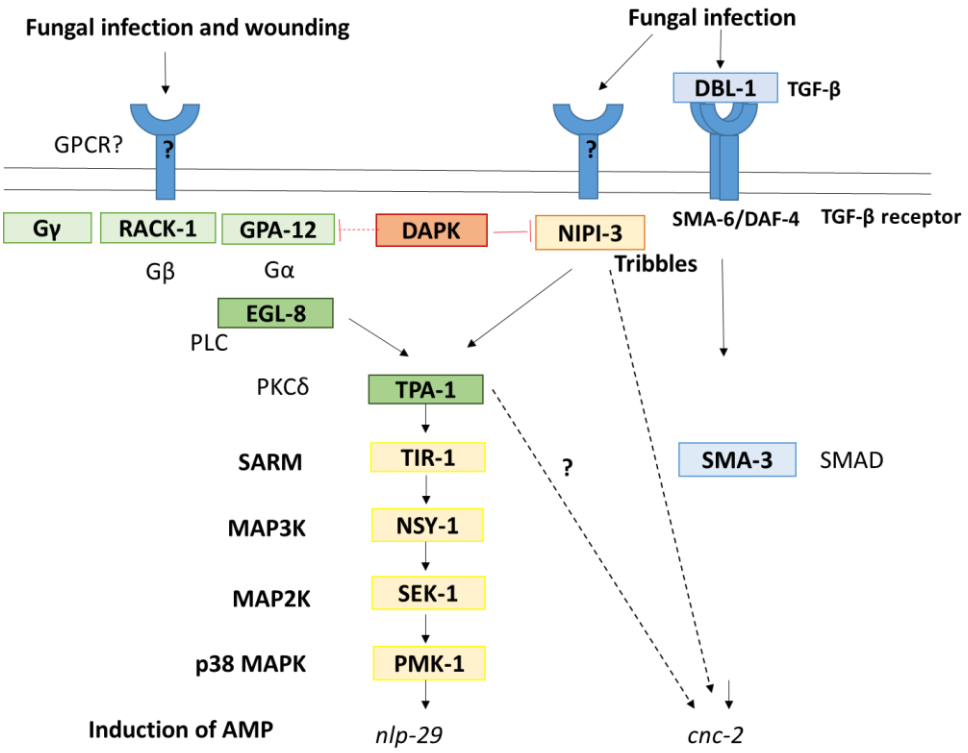


Figure 1.1. Schematic representation of various signalling pathways and their components involved in antimicrobial peptides expression induction during infection. The PKC/SARM/p38 MAPK pathway controls the expression of *nlp* genes, while TGF-b pathway controls the expression of *cnc* genes.

3.3. *C. elegans* immune response towards bacterial infections

As previously mentioned, there are shared traits within the innate immune system of humans and the nematode. The majority of the *C. elegans* immunity has been studied using nosocomial bacterial pathogens, such as *P. aeruginosa* (Kim *et al*., 2002; Irazoqui *et al*., 2010; Powell *et al*., 2009). As described by Pukkila-Worley and co-workers (2011), fungal and bacterial pathogens have remarkably distinct responses, for example, the immune response effectors shown to be up-regulated by *P. aeruginosa* are down-regulated by *C. albicans* during infection. The resistance of *C. elegans* to infection by *P. aeruginosa* triggers the DBL-1 pathway and specifically up-regulates the lysozyme gene (*lys-8*) of this pathway (Figure 1.2) (Mallo *et al*., 2002; Ewbank, 2006). The gene *dbl-1* encodes one of the four TGF-b-like ligands found in the nematode. The binding of DBL-1 to heterodimeric DAF-4/SMA-6 receptor leads to phosphorylation and function through the SMA-2/SMA-3/SMA-4 SMAD complex to regulate

gene expression. The SMA-2/SMA-3/SMA-4 complex translocate into the nucleus activating gene expression, thus linking and acting with some of the multiple isoforms of SMA-9 (zinc finger transcription factor) (Liang *et al*., 2003).

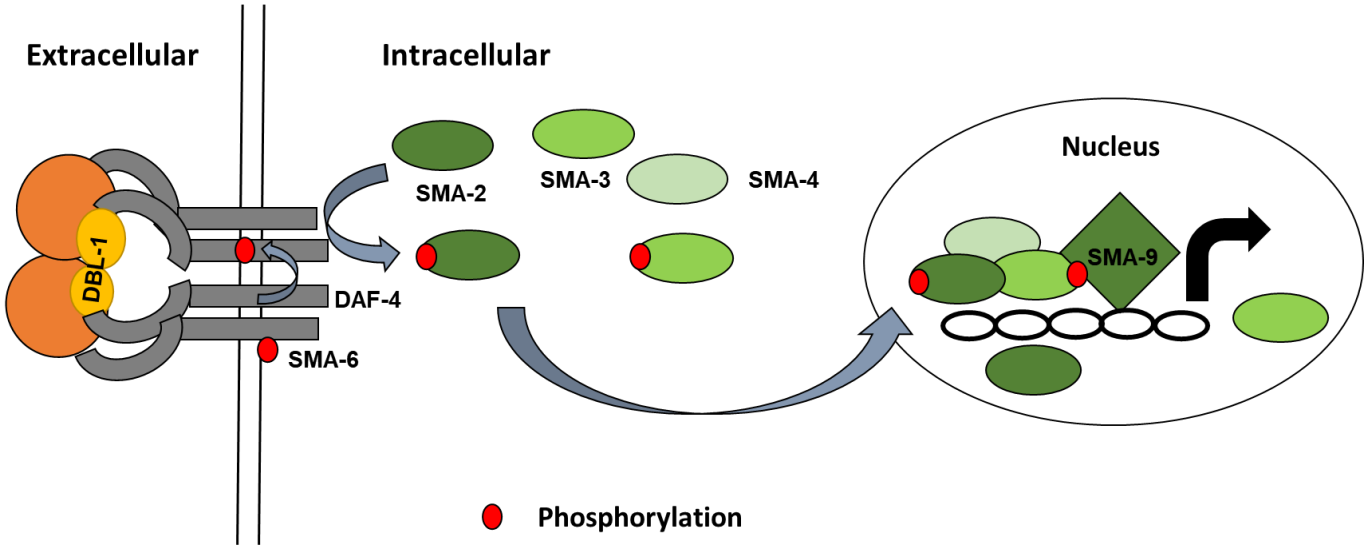


Figure 1.2. The DBL-1/TGF-b pathway. The TGF-b-like ligand binds to the heterodimeric DAF-4/SMA-6 receptor resulting to the phosphorylation and activation of the SMAD proteins SMA-2, SMA-3 and SMA-4. Thereafter the SMAD protein complex translocate into the nucleus leading to activation of gene expression, in association with SMA-9.

DAF-2/DAF-16 is another pathway involved in antibacterial defences and it highly regulates its expression (Figure 1.3) (Murphy *et al*., 2003). It is well characterized for its function of controlling longevity in *C. elegans*. Briefly, as an agonist ligand is readily present, the insulin-like peptide DAF-28 activates the DAF-2 receptor, and this activation in return activates AGE-1 phosphatidylinositol-3 OH kinase, which act as a catalyst in the conversion of phosphatidylinositol bisphosphate (PIP_2) to phosphatidylinositol trisphosphate (PIP_3) (Figure 1.3A) (Ewbank, 2006). The PIP_3 then binds to the AKT-1/AKT-2 complex, exposing the two phosphorylation sites. While also the PDK-1 kinase binds to PIP_3 recruiting it to the membrane in order to phosphorylate and activate AKT-1. Thereafter, AKT kinase phosphorylates DAF-16 transcription factor, ensuring its appropriate cytoplasmic retention. However, the presence of an antagonist ligand, INS-1, or in cases where *daf-2* lose its role, then the DAF-2/DAF-16 pathway is deactivated (Figure 1.3B) (Ewbank, 2006). This results in DAF-16 not being

phosphorylated, thus resulting to its translocation to the nucleus as it regulates the expression of coupled antimicrobial and stress response genes.

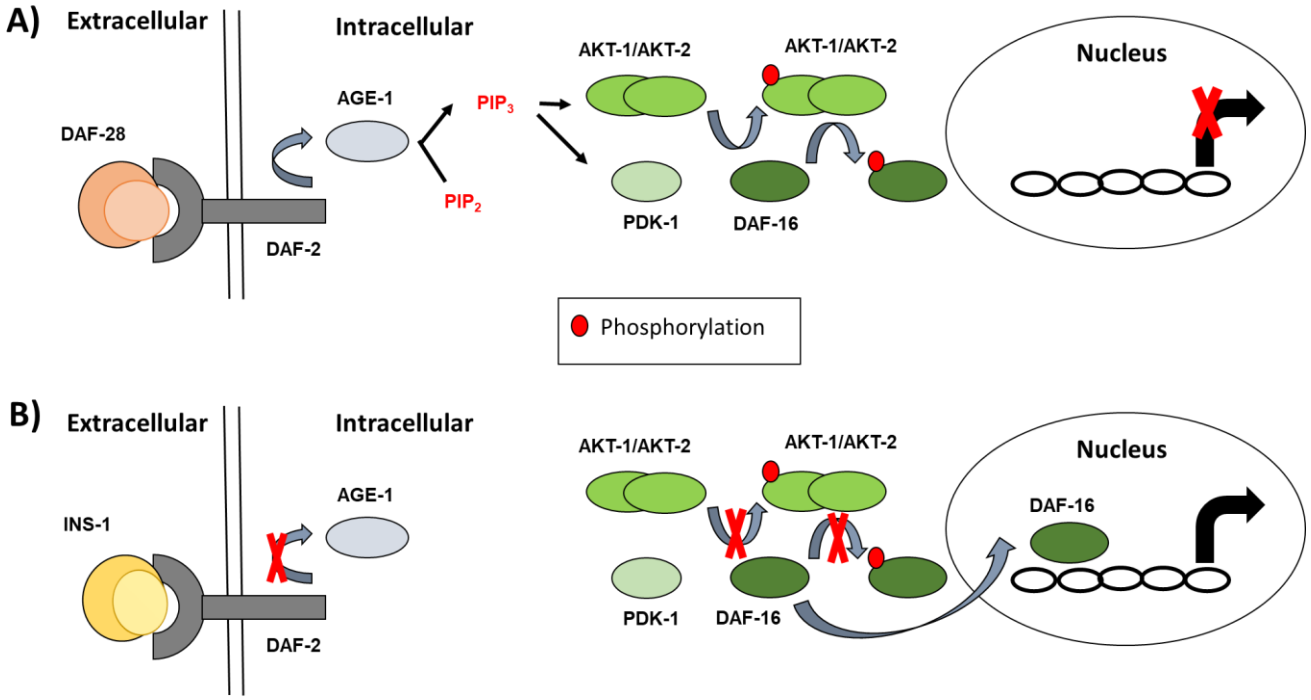


Figure 1.3. The DAF-2/DAF-16 pathway. (A) The presence of an insulin-like peptide DAF-28 agonist ligand activates the DAF-2 receptor and which in turn activates phosphatidylinositol bisphosphate (PIP_2), which is then converted into phosphatidylinositol trisphosphate (PIP_3). The AKT kinase in turn phosphorylates the DAF-16 transcription factor, ensuring its cytoplasmic retention. (B) The presence of INS-1 antagonist ligand, (or in a *daf-2* loss of function mutant), deactivates the pathway, thus DAF-16 is not phosphorylated. DAF-16 then translocate to the nucleus where it regulates the expression of coupled antimicrobial genes and stress response genes.

The resistance of *C. elegans* towards *P. aeruginosa* involves a third pathway, known as MAP kinase pathway, which involves the MAP3K NSY-1 and the MAP2K SEK-1 that was initially characterised as having a vital role in the determation of asymmetric neuronal cell fate (Kim *et al*., 2002). *nsy-1* and *sek-1* mutants, are known to express the chemoreceptor STR-2 in both the two sister olfactory neurons, while in wild-type nematodes, STR-2 is only expressed in one of the two olfactory neurons (Sagasti *et al*., 2001). Interestingly, during the genetic screening for nematodes hyper susceptible to *P. aeruginosa*, alleles of *nsy-1* and *sek-1* were

identified and shown to act together upstream of *pmk-1* (Figure 1.4). Moreover, an increased susceptibility to infection due to RNAi-mediated silencing of *pmk-1* gene, encoding one of the nematode's three p38-family MAP kinases, can also be observed (Kim *et al*., 2002). During cell determination, alleles of *nsy-1* and *sek-1* were shown to act downstream of *unc-43*, encoding a calcium-calmodulin-dependent kinase (Figure 1.4) (Kim *et al*., 2002). However, according to earlier indications this latter gene lacks a significant function in innate immune signalling (Kim *et al*., 2002). Furthermore, when RNAi abrogate the function of *pmk-1* there is no cell determination phenotype triggered, and this suggests that there are differences in the downstream components of the pathway (Chuang and Bargmann, 2005). In addition, the TIR-1 adaptor protein was proven to function in both neuronal and defence pathways. Taken together, these findings were compared with human infection, and there was a striking high degree of resemblance between *P. aeruginosa* infection in *C. elegans* and humans, hinting at a strong argument that *C. elegans* harbours features relevant to study host-pathogen interactions.

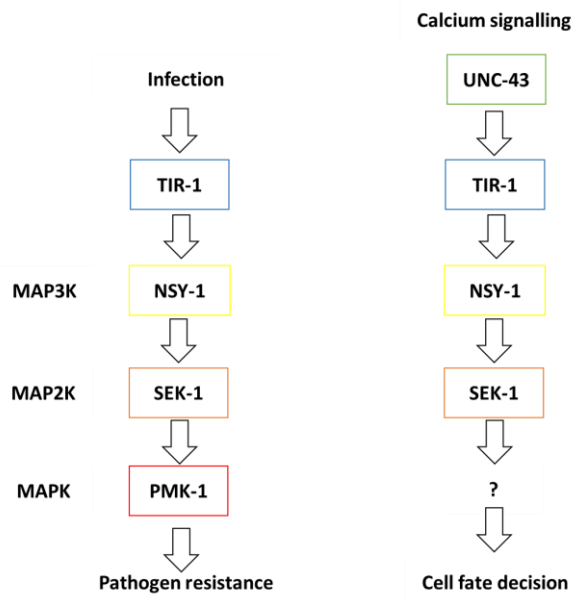


Figure 1.4. The schematic representation showing the function of *TIR-1/NSY-1/SEK-1* cassette in innate immunity and cell fate determination. During the genetic screening for nematodes hyper susceptible to *P. aeruginosa*, alleles of *nsy-1* and *sek-1* were identified and shown to act together upstream of *pmk-1.* During cell determination, alleles of *nsy-1* and *sek-1* were shown to act downstream of *unc-43*, encoding a calcium-calmodulin-dependent kinase.

3.4. Effects of lipids on immune function

In addition to the innate immune response, lipid synthesis and metabolism pathways are also largely conserved in *C. elegans* (Watts and Browse, 2002; Ashrafi *et al*., 2003; Van Gilst *et al*., 2005), therefore leading to the nematode being an ideal model to study the roles of lipids on immune function. Contrary to mammals, *C. elegans* has the ability to use its bacterial food source to synthesize all its required long chain fatty acids (LC-FAs), thus enabling the manipulation of lipid synthesis and content in the nematode (Watts and Browse, 2002; Kniazeva *et al*., 2004). The genome of *C. elegans* contains the full complement of enzymes, including elongase and desaturase enzymes, which are encoded by *elo* and *fat* genes, respectively that are required for LC-FAs synthesis (Watts and Browse, 2002; Kniazeva *et al*., 2004). The lack of apparent mammalian orthologues of cyclooxygenases and lipoxygenases or of prostanoid and leukotriene receptors in the genome of *C. elegans*, as described by Lesa *et al*. (2003), provides an opportunity to examine the effect of PUFAs in innate immunity that might be concealed by the dominanting effect of the prostaglandin and leukotriene eicosanoids.

Although lipids have numerous roles in immunity, there is fairly little evidence that exists for the specific manipulation of the lipid metabolism in response to infection. According to literature there are records of detailed analysis on the research of a whole genome microarray in *P. aeruginosa*-infected *C. elegans* gene expression showing an enrichment for genes required for the synthesis of long chains (Shapira *et al*., 2006). Therefore, this modulation of the lipid metabolism in response to infection resulted in a hint towards the potential roles of FAs in *C. elegans* immunity (Shapira *et al*., 2006). Furthermore, Nandakumar and Tan (2008) used *P. aeruginosa*-infected *C. elegans* as an infection model to investigate the interplay between PUFAs and innate immunity, in the context of the whole organism. Their findings revealed that two long chain PUFAs, gamma-linolenic acid (GLA, 18:3n-6) and stearidonic acid (SDA, 18:4n-3), are important for *C. elegans* defence against *P. aeruginosa* infection. Disruption of the biosynthesis of these two FAs resulted in increased mortality subsequent to pathogen exposure. Authors further demonstrated that through deficiency and exogenous supplementation studies, GLA and SDA are essential for both the basal expression of immunity genes and the basal activity of the p38 MAP kinase pathway (Nandakumar and Tan, 2008).

3.5. General conclusions

Although *C. elegans* has traditionally been used in molecular and developmental biology studies, over the past 20 years reserchears has been using this nematode to investigate the pathogenicity of different human bacterial (*Salmonella enterica*, *Serratia marcescens*, *P. aeruginosa* and *S. aureus*) and fungal pathogens (*Cryptococcus neoformans* and *C. albicans*) (Mylonakis *et al*., 2002; Sifri *et al*., 2003; Irazoqui *et al*., 2010; Pukkila-Worley *et al*., 2009, 2011; Sem and Rhem, 2012; Kong *et al*., 2014). Despite these pathogens being able to infect internal tissues, the transparent trait of *C. elegans* enables visualization and the study of pathogenic mechanisms of entry, replication, exit, and also host defence mechanisms within this nematode. Moreover, the early developmental events in *C. elegans* enable experimental manipulation, thus permitting for genetic and biochemical approaches in order to investigate the functions of specific lipids in pathogenicity. Thus far, studies using *C. elegans* have begun to show roles for PUFAs, eicosanoids, and endocannabinoids in development and reproduction, while the roles of these lipids during infections are still in infancy stage. Therefore, being able to understand the earliest stages of the fungal-bacterial pathogen-host interactions in *C. elegans* might provide a more complete picture of the role that lipids play during infection. Furthermore, innate immunity in nematodes is multi-tiered with physical barriers, biochemical, and genetic mechanisms to protect against pathogens. Studies revealed that many of the mechanisms involved in the human-pathogen interaction are conserved in nematodes, while other immunity mechanisms are unique to the nematode (Kim *et al*., 2002). Naturally, the usage of *C. elegans* as a model host organism is limited due to the nematode lacking adaptive immunity and its innate immunity that is less complex than higher organisms. From this point of view, the *C. elegans* model will particularly be critical for understanding complex immune signalling mechanisms involved during *C. albicans* and *P. aeruginosa* pathogenesis. Furthermore, *C. elegans* as a model will be used to determine the role of PUFAs during infection of *C. albicans* and *P. aeruginosa*.

.

3.6. Research aims and objectives

The aim of this dissertation was to determine the role of polyunsaturated fatty acids during infection of *Caenorhabditis elegans*.

The following objectives were used to accomplish the above-mentioned aim:

Objective 1

Influence of mono- and polymicrobial infection on *Caenorhabditis elegans* survival, egg retention, fatty acid composition and gene expression

Objective 2

The influence of long-chain polyunsaturated fatty acids on behaviour, susceptibility to infection and egg retention in *Caenorhabditis elegans*

Objective 3

Exploring the mechanism behind the effect of EPA supplementation on *C. albicans* infection

3.7. References

Aballay, A. & Ausubel. F. M. (2002). *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. *Curr Opin Microbiol* **5**, 97–101.

Alper, S., McBride, S. J., Lackford, B., Freedman, J. H. & Schwartz, D. A. (2007). Specificity and complexity of the *Caenorhabditis elegans* innate immune response. *Mol Cell Biol* **27** (15), 5544–5553.

Anderson, S. M., Cheesman, H. K., Peterson, N. D., Salisbury, J. E., Soukas, A. A. & Pukkila-Worley, R. (2019). The fatty acid oleate is required for innate immune activation and pathogen defence in *Caenorhabditis elegans*. *PLoS Pathogens* **15** (6).

Artal-Sanz, M., de Jong, L. & Tavernarakis, N. (2006). *Caenorhabditis elegans*: a versatile platform for drug discovery. *Biotechnol J* **1**, 1405–1418.

Ashrafi, K., Chang, F. Y., Watts, J. L., Fraser, A. G., Kamath, R. S. *et al*. (2003). Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* **421**, 268–72.

Barrière, A. & Felix, M. A. (2006). Isolation of *C. elegans* and related nematodes. The *C. elegans* Research Community: WormBook.

Bauernfeind, A., Bertele, R. M., Harms, K., Horl, G., Jungwirth, R., Petermuller, C., Przyklenk, B. & Weisslein-Pfister, C. (1987). Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection* **15**, 270–277.

Bergeron, A. C., Seman, B. G., Hammond, J. H., Archambault, L. S., Hogan, D. A. & Wheeler, R. T. (2017). *Candida* and *Pseudomonas* interact to enhance virulence of mucosal infection in transparent zebrafish. *Infect Immun* **85**.

Botos, I., Segal, D. M. & Davies, D. R. (2011). The structural biology of Toll-like receptors. *Structure* **19** (4), 447–459.

Brandt, J. P. & Ringstad, N. (2015). Toll-like receptor signaling promotes development and function of sensory neurons required for a *C. elegans* pathogen-avoidance behavior. *Curr Biol* **25** (17), 2228-2237.

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M. & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog* **3**, e18.

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

Brock, T. J., Browse, J. & Watts, J. L. (2006). Genetic regulation of unsaturated fatty acid composition in *C. elegans*. *PLoS Genet* **2**, e108.

Burns, A. R., Kwok, T. C., Howard, A., Houston, E., Johanson, K., Chan, A. *et al*. (2006). High-throughput screening of small molecules for bioactivity and target identification in *Caenorhabditis elegans*. *Nat Protoc* **1**, 1906–1914.

Burns, J. L., Van Dalfsen, J. M., Shawar, R. M., Otto, K. L., Garber, R. L., Quan, J. M., Montgomery, A. B., Albers, G. M., Ramsey, B. W. & Smith, A. L. (1999). Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis* **179**, 1190–1196.

Calderone, R. A. & Fonzi, W. A. (2001). Virulence factors of *Candida albicans*. *Trends Microbiol* **9**, 327–335.

Catalá, A. (2010). A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. *Biochem Biophys Res Commun* **399**, 318-323.

Chandra, J., Kuhn, D. M., Muckerjee, P. K., Hoyer, L. L., McCormick, T. & Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol* **183**, 5385-5394.

Chisholm, A. D. & Hsiao, T. I. (2012). The *Caenorhabditis elegans* epidermis as a model skin. I: development, patterning, and growth. *Wires Dev Biol* **1**, 861–878.

Chuang, C. F. & Bargmann, C. I. (2005). A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev* **19**, 270-281.

Cohen, L. B. & Troemel, E. R. (2015). Microbial pathogenesis and host defense in the nematode *C. elegans*. *Curr Opin Microbiol* **23**, 94-101.

Corsi, A. K., Wightman, B. & Chalfie, M. (2015). A transparent window into biology: A primer on *Caenorhabditis elegans*. *Genetics* **200**, 387–407.

Costa-Orlandi, C. B., Sardi, J. C. O., Pitangui, N. S., de Oliveira H. C., Scorzoni, L., Galeane, M. C., Medina-Alarcón, K. P., Melo, W. C. M. A., Marcelino, M. Y. *et al*. (2017). Fungal biofilms and polymicrobial diseases. *J Fungi* **3**, 22.

Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J. F. *et al*. (2004). TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat Immunol* **5**, 488–494.

Diaz, P. I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., Ikonomou, L. & Dongari-Bagtzoglou, A. (2012). Synergistic interaction between *Candida albicans* and commensal oral streptococci in a novel *in vitro* mucosal model. *Infect Immun* **80** (2), 620–632.

Darby, C., Hsu, J. W., Ghori, N. & Falkow, S. (2002). *Caenorhabditis elegans*: plague bacteria biofilm blocks food intake. *Nature* **417**, 243–244.

Das, U. N. (2018). Arachidonic acid and other unsaturated fatty acids and some of their metabolites function as endogenous antimicrobial molecules: a review. *J Adv Res* **11**, 57-66.

Dasgupta, M., Shashikanth, M., Gupta, A., Sandhu, A., De, A., Javed, S. & Singh, V. (2020). NHR-49 transcription factor regulates immunometabolic response and survival of *Caenorhabditis elegans* during *Enterococcus faecalis* infection. *Infect Immun* **88** (8), e00130-20.

Deline, M. L., Vrablik, T. L. & Watts, J. L. (2013). Dietary supplementation of polyunsaturated fatty acids in *Caenorhabditis elegans*. *J Vis Exp* **81**, 50879.

de Macedo, J. L. & Santos, J. B. (2005). Bacterial and fungal colonization of burn wounds. *Mem Inst Oswaldo Cruz* **100**, 535–539.

Dijksterhuis, J., Veenhuis, M. & Harder, W. (1990). Ultrastructural study of adhesion and initial stages of infection of the nematode by conidia of *Drechmeria coniospora. Mycol Res* **94**, 1–8.

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends Microbiol* **11**, 30–36.

Durai, S., Vigneshwari, L. & Balamurugan, K. (2013). *Caenorhabditis elegans*-based *in vivo* screening of bioactives from marine sponge-associated bacteria against *Vibrio alginolyticus*. *J Appl Microbiol* **115**, 1329–1342.

Eng, S. A. & Nathan, S. (2015). Curcumin rescues *Caenorhabditis elegans* from a *Burkholderia pseudomallei* infection. *Front Microbiol* **6**, 290.

Erb-Downward, J. R. & Huffnagle, G. B. (2007). *Cryptococcus neoformans* produces authentic prostaglandin E_2 without a cyclooxygenase. *Eukaryot Cell* **6**, 346–350.

Ewbank, J. J. (2006). Signaling in the immune response. *WormBook: the online review of C. elegans biology* 1-12.

Funk, C. D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* **294**, 1871–1875.

Garsin, D. A., Villanueva, J. M., Begun, J., Kim, D. H., Sifri, C. D., Calderwood, S. B., Ruvkun, G. & Ausubel, F. M. (2003). Long-lived *C. elegans* daf-2 mutants are resistant to bacterial pathogens. *Science* **300** (5627), 1921.

Gilst, M. R. V., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005). Nuclear hormone receptor nhr-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* **3**, e53.

Gow, N. A. (1997). Germ tube growth of *Candida albicans*. *Curr Top Med Mycol* **8**, 43–55.

Gow, N. A., Brown, A. J. & Odds, F. C. (2002). Fungal morphogenesis and host invasion. *Curr Opin Microbiol* **5**, 366–371.

Gow, N. A. R. & Hube, B. (2012). Importance of the *Candida albicans* cell wall during commensalism and infection. *Curr Opin Microbiol* **15**, 406–412.

Gow, N. A., Van De Veerdonk, F. L., Brown, A. J. & Netea, M. G. (2012). *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* **10**, 112–122.

Gratacap, R. L., Bergeron, A. C. & Wheeler, R. T. (2014). Modeling mucosal candidiasis in larval Zebrafish by swimbladder injection. *J Vis Exp* **93**, e52182.

Gupta, N., Haque, A., Mukhopadhyay, G., Narayan, R. P. & Prasad, R. (2005). Interactions between bacteria and *Candida* in the burn wound. *Burns* **31**, 375–378.

Harriot, M. M. & Noverr, M. C. (2009). *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrob Agents Chemother* **53**, 3914-3922.

Hermann, C., Hermann, J., Munzel, U. & Rüchel, R. (1999). Bacterial flora accompanying *Candida* yeasts in clinical specimens. *Mycoses* **42** (11-12), 619–627.

Hodgkin, J., Kuwabara, P. E. & Corneliussen, B. (2000). A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans. Curr Biol* **10** (24), 1615-8.

Hogan, D. A. & Kolter, R. (2002). *Pseudomonas-Candida* interactions: an ecological role for virulence factors. *Science* **296**, 2229-2232.

Hube, B. (2006). Infection-associated genes of *Candida albicans. Future Microbiol* **1**, 209–218.

Hughes, W. T. & Kim, H. K. (1973). Mycoflora in cystic fibrosis: some ecologic aspects of *Pseudomonas aeruginosa* and *Candida albicans*. *Mycopathol Mycol Appl* **50**, 261–269.

Irazoqui, J. E., Troemel, E. R., Feinbaum, R. L., Luhachack, L. G., Cezairliyan, B. O. & Ausubel, F. M. (2010). Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. *PLoS Pathog* **6**, e1000982.

Jia, X., Xu, M., Yang, A., Zhao, Y., Liu, D., Huang, J., Proksch, P. & Lin, W. (2019). Reducing effect of farnesylquinone on lipid mass in *C. elegans* by modulating lipid metabolism. *Mar Drugs* **17**, 336.

Kaleli, I., Cevahir, N., Demir, M., Yildirim, U. & Sahin, R. (2007). Anticandidal activity of *Pseudomonas aeruginosa* strains isolated from clinical specimens. *Mycoses* **50**, 74–78.

Kato, Y., Aizawa, T., Hoshino, H., Kawano, K., Nitta, K. & Zhang, H. (2002). abf-1 and abf-2, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans. Biochem J* **361**, 221–230.

Kerr, J. (1994). Inhibition of fungal growth by *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from patients with cystic fibrosis. *J Infect* **28**, 305-310.

Kim, D. H., Feinbaum, R., Alloing, G., Emerson, F. E., Garsin, D. A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M. W. & Ausubel, F. M. (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623-626.

Kim, D., Liu, Y., Benhamou, R. I., Sanchez, H., Simón-Soro, A., Li, Y., Hwang, G., Fridman, M., Andes, D. R. *et al*. (2018). Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm. *ISME J* **12**, 1427-1442.

Kniazeva, M., Crawford, Q. T, Seiber, M., Wang, C. Y. & Han, M. (2004). Monomethyl branched-chain fatty acids play an essential role in *Caenorhabditis elegans* development. *PLoS Biol* **2**, e257.

Kohn, A., Gitelman, J. & Inbar, M. (1980). Unsaturated free fatty acids inactivate animal enveloped viruses. *Arch Virol* **66**, 301-306.

Kong, C., Eng, S. A., Lim, M. P. & Nathan, S. (2016). Beyond traditional antimicrobials: a *Caenorhabditis elegans* model for discovery of novel anti-infectives. *Front microbiol* **7**, 1956.

Kong, C., Yehye, W. A., Abd Rahman, N., Tan, M. W. & Nathan, S. (2014). Discovery of potential anti-infectives against *Staphylococcus aureus* using a *Caenorhabditis elegans* infection model. *BMC Complement Altern Med* **14**, 4.

Kulshreshtha, G., Borza, T., Rathgeber, B., Stratton, G. S., Thomas, N. A., Critchley, A., Hafting, J. & Prithiviraj, B. (2016). Red seaweeds *Sarcodiotheca gaudichaudii* and *Chondrus crispus* down regulate virulence factors of *Salmonella Enteritidis* and induce immune responses in *Caenorhabditis elegans*. *Front Microbiol* **7**, 421.

Kurz, C. L. & Ewbank, J. J. (2003). *Caenorhabditis elegans*: an emerging genetic model for the study of innate immunity. *Nat Rev Genet* **4** (5), 380-90.

Labrousse, A., Chauvet, S., Couillault, C., Kurz, C. L. & Ewbank, J. J. (2000). *Caenorhabditis elegans* is a model host for *Salmonella typhimurium*. *Curr Biol* **10** (23), 1543-5.

Lemaitre, B., Reichhart, J. –M. & Hoffmann, J. A. (1997). *Drosophila* host defense: Differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. *Proc Natl Acad Sci USA* **94**, 14614–14619.

Lesa, G. M., Palfreyman, M., Hall, D. H., Clandinin, M. T., Rudolph, C., Jorgensen, E. M. & Schiavo, G. (2003). Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans. J Cell Sci* **116**, 4965–4975.

Liberati, N. T., Fitzgerald, K. A., Kim, D. H., Feinbaum, R., Golenbock, D. T. & Ausubel, F. M. (2004). Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *Proc Natl Acad Sci USA* **101** (17), 6593-8.

Liang, J., Lints, R., Foehr, M. L., Tokarz, R., Yu, L., Emmons, S. W., Liu, J. & Savage-Dunn, C. (2003). The *Caenorhabditis elegans schnurri* homolog sma-9 mediates stage- and cell type-specific responses to DBL-1 BMP-related signaling. *Development* **130**, 6453–6464.

Lindsay, A. K. & Hogan, D. A. (2014). *Candida albicans*: molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Fungal Biol Rev* **28**, 85-96.

Liu, H. (2002). Co-regulation of pathogenesis with dimorphism and phenotypic switching in *Candida albicans*, a commensal and a pathogen. *Int J Med Microbiol* **292**, 299–311.

Lohse, M. B., Gulati, M., Johnson, A. D. & Nobile, C. J. (2018). Development and regulation of single- and multi-species *Candida albicans* biofilms. *Nat Rev Microbiol* **16**, 19-31.

Mallo, G. V., Kurz, C. L., Couillault, C., Pujol, N., Granjeaud, S., Kohara, Y. & Ewbank, J. J. (2002). Inducible antibacterial defense system in *C. elegans*. *Curr Biol* **12**, 1209–1214.

Massey K. A. & Nicolaou A. (2011). Lipidomics of polyunsaturated-fatty-acid-derived oxygenated metabolites. *Biochem Soc Trans* **39**, 1240–1246.

McAlester, G., O'Gara, F. & Morrissey, J. P. (2008). Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. *J Med Microbiol* **57**, 563–569.

Méar, J. B., Kipnis, E., Faure, E., Dessein, R., Schurtz, G., Faure, K. & Guery, B. (2013). *Candida albicans* and *Pseudomonas aeruginosa* interactions: more than an opportunistic criminal association? *Méd Mal Infect* **43**,146-151.

Mokoena, N. Z., Sebolai, O. M., Albertyn, J. & Pohl, C. H. (2020). Synthesis and function of fatty acids and oxylipins, with a focus on *Caenorhabditis elegans. Prostag Oth Lipid M* **148**, 106426.

Moy, T. I., Ball, A. R., Anklesaria, Z., Casadei, G., Lewis, K. & Ausubel, F. M. (2006). Identification of novel antimicrobials using a live-animal infection model. *Proc Natl Acad Sci USA* **103**, 10414–10419.

Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., Li, H. & Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**, 277–283.

Murray, J. L., Connell, J. L., Stacy, A., Turner, K. H. & Whiteley, M. (2014). Mechanisms of synergy in polymicrobial infections. *J Microbiol* **52**, 188-199.

Mylonakis, E., Ausubel, F. M., Perfect, J. R., Heitman, J. & Calderwood, S. B. (2002). Killing of *Caenorhabditis elegans* by *Cryptococcus neoformans* as a model of yeast pathogenesis. *Proc Natl Acad Sci USA* **99**, 15675–15680.

Naglik, J., Albrecht, A., Bader, O. & Hube, B. (2004). *Candida albicans* proteinases and host/pathogen interactions. *Cell Microbiol* **6**, 915–926.

Nandakumar, M. & Tan, M. W (2008). Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity. *PLoS Genet* **4** (11), e1000273.

Neely, A. N., Law, E. J. & Holder, I. A. (1986). Increased susceptibility to lethal *Candida* infections in burned mice preinfected with *Pseudomonas aeruginosa* or pretreated with proteolytic enzymes. *Infect Immun* **52**, 200–204.

Noverr, M. C., Phare, S. M., Toews, G. B., Coffey, M. J. & Huffnagle, G. B. (2001). Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect Immun* **69**, 2957-2963.

Oever, J. T. & Netea, M. G. (2014). The bacteriome–mycobiome interaction and antifungal host defense. *Eur J Immunol* **44**, 3182–3191.

O'Neil, D. A., Porter, E. M., Elewaut, D., Anderson, G. M., Eckmann, L. *et al*. (1999). Expression and regulation of the human {beta}-defensins hBD-1 and hBD-2 in intestinal epithelium. *J Immunol* **163**, 6718–6724.

Ooi, J. Y., Yagi, Y., Hu, X. & Ip, Y. T. (2002). The *Drosophila* Toll-9 activates a constitutive antimicrobial defense. *EMBO Rep* **3**, 82–87.

Palmer, K. L., Kos, V. N. & Gilmore, M. S. (2010). Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr Opin Microbiol* **13**, 632–9.

Peleg, A. Y., Tampakakis, E., Fuchs, B. B., Eliopoulos, G. M., Moellering, R. C. *et al*. (2008). Prokaryote-eukaryote interactions identified by using *Caenorhabditis elegans. Proc Natl Acad Sci USA* **105**, 14585-14590.

Peng, D., Luo, X., Zhang, N., Guo, S., Zheng, J., Chen, L. & Sun, M. (2018). Small RNA-mediated Cry toxin silencing allows *Bacillus thuringiensis* to evade *Caenorhabditis elegans* avoidance behavioral defenses. *Nucleic Acids Res* **46** (1), 159-173.

Peters, B. M., Jabra-Rizk, M. A., O'May, G. A., Costerton, J. W. & Shirtliff, M. E. (2012). Polymicrobial interactions: impact on pathogenesis and human disease. *Clin Microbiol Rev* **25**, 193-213.

Pfaller, M. A. & Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* **20**, 133-163.

Pohl, C. H., Kock, J. L. F. & Thibane, V. S. (2011). Antifungal free fatty acids: a review. In *Science against Microbial Pathogens*: *Communicating Current Research and Technological Advances*, Méndez-Vilas. A. (ed) Spain: Formatex Research Center **1**, 61-71.

Powell, J. R. & Ausubel, F. M. (2008). Models of *Caenorhabditis elegans* infection by bacterial and fungal pathogens. *Methods Mol Biol* **415**, 403–427.

Powell, J. R, Kim, D. H. & Ausubel, F. M. (2009). The G protein-coupled receptor FSHR-1 is required for the *Caenorhabditis elegans* innate immune response. *Proc Natl Acad Sci USA* **106**, 2782–2787.

Pradel, E., Zhang, Y., Pujol, N., Matsuyama, T., Bargmann, C. I. & Ewbank, J. J. (2007). Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **104** (7), 2295–2300.

Pujol, N., Cypowyj, S., Ziegler, K., Millet, A., Astrain, A. *et al*. (2008). Distinct innate immune responses to infection and wounding in the *C. elegans* epidermis. *Curr Biol* **18**, 481–489.

Pujol, N., Link, E. M., Liu, L. X., Kurz, C. L., Alloing, G., Tan, M. W., Ray, K. P., Solari, R., Johnson, C. D. & Ewbank, J. J. (2001). A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. *Curr Biol* **11** (11), 809-21.

Pukkila-Worley, R., Ausubel, F. M. & Mylonakis, E. (2011). *Candida albicans* infection of *Caenorhabditis elegans* induces antifungal immune defences. *PLoS Pathog* **7** (6), e1002074.4.

Pukkila-Worley, R., Peleg, A. Y., Tampakakis, E. & Mylonakis, E. (2009). *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryot Cell* **8**, 1750–1758.

Roux, D., Gaudry, S., Dreyfuss, D., El-Benna, J., de Prost, N., Denamur, E., Saumon, G. & Ricard, J. (2009). *Candida albicans* impairs macrophage function and facilitates *Pseudomonas aeruginosa* pneumonia in rat. *Crit Care Med* **37**, 1062–1067.

Sagasti, A., Hisamoto, N., Hyodo, J., Tanaka-Hino, M., Matsumoto, K. & Bargmann, C. I. (2001). The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* **105**, 221–232.

Sandhu, A., Badal, D., Sheokand, R., Tyagi, S. & Singh, V. (2021). Specific collagens maintain the cuticle permeability barrier in *Caenorhabditis elegans. Genetics* **217** (3), iyaa047.

Sem, X. & Rhen, M. (2012). Pathogenicity of *Salmonella enterica* in *Caenorhabditis elegans* relies on disseminated oxidative stress in the infected host. *PLoS ONE* **7**.

Schmid-Hempel, P. & Ebert, D. (2003). On the evolutionary ecology of specific immune defence. *Trends Ecol Evol* **18**, 27–32.

Shtonda, B. B. & Avery, L. (2006). Dietary choice behaviour in *Caenorhabditis elegans. J Exp Biol* **209**, 89–102.

Schulenburg, H., Kurz, C. L. & Ewbank, J. J. (2004). Evolution of the innate immune system: the worm perspective. *Immunol Rev* **198**, 36–58.

Shapira, M., Hamlin, B. J., Rong, J., Chen, K., Ronen, M. & Tan, M. W. (2006). A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc Natl Acad Sci USA* **103** (38), 14086-91.

Sifri, C. D., Begun, J., Ausubel, F. M. & Calderwood, S. B. (2003). *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. *Infect Immun* **71**, 2208–2217.

Sim, S. & Hibberd, M. L. (2016). *Caenorhabditis elegans* susceptibility to gut *Enterococcus faecalis* infection is associated with fat metabolism and epithelial junction integrity. *BMC microbiol* **16**, 6.

Stables, M. J. & Gilroy, D. W. (2011). Old and new generation lipid mediators in acute inflammation and resolution. *Prog Lipid Res* **50**, 35–51.

Stacy, A., McNally, L., Darch, S. E., Brown, S. P. & Whiteley, M. (2016). The biogeography of polymicrobial infection. *Nat Rev Microbiol* **14**, 93-105.

Tallima, H. & El Ridi, R. (2018). Arachidonic acid: Physiological roles and potential health benefits – A review. *J Adv Res* **11**, 33-41.

Trejo-Hernández, A., Andrade-Domínguez, A., Hernández, M. & Encarnación, S. (2014). Interspecies competition triggers virulence and mutability in *Candida albicans–Pseudomonas aeruginosa* mixed biofilms. *ISME J* **8**, 1974-1988.

Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M. & Kim, D. H. (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* **2** (11), e183.

Troemel, E. R., Felix, M. A., Whiteman, N. K., Barriere, A. & Ausubel, F. M. (2008). *Microsporidia* are natural intracellular parasites of the nematode *Caenorhabditis elegans*. *PLoS Biol* **6**, e309.

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* **3**, e53.

Vrablik, T. L. & Watts, J. L. (2013). Polyunsaturated fatty acid derived signaling in reproduction and development: insights from *Caenorhabditis elegans* and *Drosophila melanogaster*. *Mol Reprod Dev* **80**, 244–59.

Wahli, W. & Michalik, L. (2012). PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol Metab* **23**, 351–63.

Wallis, J. G., Watts, J. L. & Browse, J. (2002). Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem Sci* **27**, 467.

Watts, J. L. (2016). Using *Caenorhabditis elegans* to uncover conserved functions of omega-3 and omega-6 fatty acids. *J Clin Med* **5**, e19.

Watts, J. L. & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **99**, 5854–5859.

Watts, J. L., Phillips, E., Griffing, K. R. & Browse, J. (2003). Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *Caenorhabditis elegans fat-3* mutants. *Genetics* **163**, 581–589.

Wes, P. D. & Bargmann, C. I. (2001). *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. *Nature* **410** (6829), 698-701.

Whiteway, M. & Oberholzer, U. (2004). *Candida* morphogenesis and host-pathogen interactions. *Curr Opin Microbiol* **7**, 350–357.

Wolcott, R., Costerton, J. W., Raoult, D. & Cutler, S. J. (2013). The polymicrobial nature of biofilm infection. *Clin Microbiol Infect* **19**, 107-112.

Xu, L., Wang, F., Shen, Y., Hou, H., Liu, W., Liu, C. *et al*. (2014). *Pseudomonas aeruginosa* inhibits the growth of pathogenic fungi: *in vitro* and *in vivo* studies. *Exp Ther Med* **7**, 1516-1520.

Yi, Y. H., Chien, C. -H., Chen, W. -W., Ma, T. -H., Liu. K. -Y., Chang, Y. -S., Chang, T. -C. & Lo, S. J. (2014). Lipid droplet pattern and nondroplet-like structure in two fat mutants of *Caenorhabditis elegans* revealed by coherentanti-Stokes Raman scattering microscopy. *J Biomed Opt* **19**, 011011.

Yoon, B. K., Jackman, J. A., Valle-Gonzáles, E. R. & Cho, N. -J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int J Mol Sci* **19**, E1114.

Zhao, C., Wang, I. & Lehrer, R. I. (1996). Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. *FEBS Lett* **396**, 319–322.

Ziegler, K., Kurz, C. L., Cypowyj, S., Couillault, C., Pophillat, M., Pujol, N. & Ewbank, J. J. (2009). Antifungal innate immunity in *C. elegans*: PKCdelta links G protein signaling and a conserved p38 MAPK cascade. *Cell Host Microbe* **5** (4), 341-52.

Chapter 2

Influence of mono- and polymicrobial infection on *Caenorhabditis elegans* survival, egg retention, fatty acid composition and gene expression

1. Abstract

Although virulence is a critical component of infection outcome, our current understanding of how virulence influences polymicrobial infection remains minimal. Infection models that recapitulate mammalian infection, but allow simultaneous monitoring of host, bacteria and fungi may bridge this knowledge gap. The juvenile *Caenorhabditis elegans* model offers the power to track fungal, bacterial, and host dynamics simultaneously and at high resolution over the course of a live infection with the ability to longitudinally associate infection factors with mortality. Therefore, we sought to investigate the interaction between *Candida albicans* and *Pseudomonas aeruginosa* using the *C. elegans* killing liquid media assay. The survival of the infected nematodes was significantly reduced compared to uninfected nematodes, with polymicrobial infection resulting in a synergistically more virulent co-infection. Following investigation of the ability of *C. elegans* to retain eggs when exposed to pathogens, we uncovered that the degree of egg retention elicited by *P. aeruginosa* and *C. albicans* varied and was similarly increased by polymicrobial infection. Finally, using gas chromatography for fatty acid (FA) analysis, we studied the influence of infection on the FA composition of nematodes, as well as the expression of genes involved in lipid metabolism and immune response. All infected nematodes showed changes in FA composition with a decrease in the unsaturation index of the nematodes lipids compared to control. There was also a correlation between the observed virulence of the infections and the level of decrease in the unsaturation index of the FAs. These changes were corroborated by the observed changes in expression of lipid metabolism genes. Lastly, we observed down-regulation of the nematode innate immunity effectors after infection. These findings present a promising extension to ongoing research in the study of monomicrobial pathogenesis using *C. elegans* and provides new information in terms of an animal model used in studying multifaceted dynamics of polymicrobial infection and involvement of FAs.

Key word: *Caenorhabditis elegans*, *Candida albicans*, *Pseudomonas aeruginosa*, monomicrobial, polymicrobial, polyunsaturated, fatty acids, gas chromatography, gene expression

2. Introduction

During polymicrobial infection, interactions between various pathogenic microorganisms often alter microbial virulence, pathogenesis and host responses, thus resulting in complications during treatment of a patient and resolution of infection. *Candida albicans* is one of the most commonly isolated fungi in the co-infection between fungi and bacteria (Valenza *et al*., 2008; Kim *et al*., 2015). This pathogen is a dimorphic opportunist that possess the ability to form invasive hyphal filaments and drug-resistant biofilms (Chandra *et al*., 2001; Sudbery *et al*., 2004). The virulence of *C. albicans* is influenced by its ability to shift from yeast to hyphal forms, controlled by numerous cues including quorum sensing (Naglik *et al*., 2003; Moyes and Naglik, 2011; Mayer *et al*., 2013). *Pseudomonas aeruginosa* is another opportunistic pathogen with sophisticated virulence factors, such as biofilm formation and the production of toxins (Bomberger *et al*., 2009; Balasubramanian *et al*., 2013). Alarmly, this bacteria is associated with nosocomial infections with a relatively high mortality rate (Moy *et al*., 2006; Driscoll *et al*., 2007). *Pseudomonas aeruginosa* can co-exist with *C. albicans* in hospitalised patients, including cystic fibrosis patients, resulting in polymicrobial infections and complications in administering therapies (Pierce, 2005; Krüger *et al*., 2019). Indeed, several lines of evidence suggest that *C. albicans* and *P. aeruginosa* can interact directly in a contact-dependent fashion or indirectly via inhibition of virulence factors *in vitro* (Lindsay and Hogan, 2014; Xu *et al*., 2014b; Fourie *et al*., 2016; Fourie and Pohl, 2019), although the relevance of these interactions remains controversial in animal models. For instance, research executed in some *C. albicans*-bacterial co-infection murine models have shown a synergistic virulence for one or both pathogens, thus suggesting that the outcome of co-infections is not easily predicted from *in vitro* antagonism experiments (Xu *et al*., 2014b; Nash *et al*., 2014, 2015; Schlecht *et al*., 2015; Pendleton *et al*., 2017; Fourie and Pohl, 2019). In a murine burn model, it was discovered that pre-infection with *P. aeruginosa* has the ability to increase the damage caused by *C. albicans* (Neely *et al*., 1986). However, some studies involving co-infections with *C. albicans* and *P. aeruginosa* in murine and *Caenorhabditis elegans* models found that the combination of these two pathogens can enhance overall virulence in the context of co-infection (Peleg *et al*., 2008; Lopez-Medina *et al*., 2015). These disparities suggest that other factors, including the host environment, can play a role in the way bacteria and fungi interact.

To address the lack of functional evidence regarding model-based polymicrobial infections, we have utilised the genetically tractable nematode *C. elegans*, a commonly used model for understanding microbial host-pathogen interactions (Garsin *et al*., 2001; Aballay and Ausubel, 2002; Kirienko *et al*., 2013). Since the nematode is a bacterivore, and therefore constantly exposed to various pathogens (Corsi *et al*., 2015), this makes it easy to establish intestinal infection through feeding, in order to investigate the virulence mechanism adopted by human

pathogens (Tan *et al*., 1999; Pukkila-Worley *et al.*, 2009). Additionally, the other experimental advantages that make *C. elegans* more amenable for the study of microbial pathogenesis include simple growth conditions, transparent body, rapid generation time, short body length, large brood size, short lifespan and completely sequenced genome (Corsi *et al*., 2015). Thus, in this present study, we aimed to investigate the influence of *C. albicans* and *P. aeruginosa* polymicrobial infections on *C. elegans*. The effects monitored included survival and stress response, using egg retention as marker. We also studied the influence of infections on fatty acid (FA) composition of *C. elegans*, given that lipids are also involved in fungal growth and pathogenicity (Noverr *et al*., 2001; Shea *et al*., 2006; Erb-Downward and Huffnagle, 2007; Rhome and Del Poeta, 2010). For instance, *C. albicans,* in concert with other microorganisms such as bacteria, may be influenced by immunomodulatory FA metabolites produced by both the host and bacterial co-colonisers (Lindsay and Hogan, 2014). Although there have been intensive studies done on the function of lipid mediators between monomicrobial pathogenic species and hosts, little evidence still exists concerning their function during polymicrobial infection of *C. albicans* and *P. aeruginosa* and towards the host. Therefore, this requires further investigations to gain full insight regarding the *C. albicans* and *P. aeruginosa* polymicrobial interaction in context of FAs and gene expression, and the role of these FAs in a host, such as *C. elegans*, during infection and co-infection.

3. Materials and methods

3.1. *Caenorhabditis elegans* strain and culture conditions

Caenorhabditis elegans glp-4; sek-1 hermaphrodites, obtained from the *Caenorhabditis* Genetic Centre, College of Biological Sciences, University of Minnesota, were propagated on Nematode Growth Medium (NGM) (2.5 g/L peptone, 3 g/L sodium chloride, 17 g/L agar) spotted with *Escherichia coli* OP50 (a uracil-requiring mutant of *E. coli*). The rationale behind the use of *C. elegans glp-4*; *sek-1* mutant as a replacement for wild type nematodes, is due to the fact that the *C. elegans* wild type is capable of producing many offspring which can confound killing assays. This can be challenging when separating adults from progeny. Moreover, larvae can hatch inside the nematode, killing the nematode through a process called matricidal killing, which is not directly related to pathogen pathogenicity (Breger *et al*., 2007; Peleg *et al*., 2008; Pukkila-Worley *et al*., 2009). In contrast to wild types, *glp-4* mutants cannot produce gonads or progeny at 25 °C, making it beneficial for these studies (Miyata *et al*., 2008). Since *C. elegans sek-1* lack a gene that encodes a conserved mitogen-activated protein kinase (MAPKK) involved in the innate immune response, these nematodes are thus

immunocompromised, allowing them to become infected with various pathogens (Kim *et al*., 2002).

About 10 to 15 reproductively active, synchronised *C. elegans* adult hermaphrodites were moved to fresh NGM agar plate and incubated at 15 °C to allow the nematode to lay eggs. After laying of eggs, all adult nematodes were removed from plate. Then, the eggs remaining on the agar plates were allowed to hatch, and allowed to develop to L4 larvae at 15 °C. Thereafter, approximately 20 larva 4 (L4) were moved to a fresh NGM agar plate with *E. coli* OP50 lawn. This is followed by incubation of NGM agar plates with nematodes at 15 °C to allow nematodes to propagate and feed on all the bacterial food. *Caenorhabditis elegans* nematodes were transferred to new NGM agar plates seeded with lawn of *E. coli* OP50 every 6 days. All *C. elegans* stocks were kept at 15 °C until further use. All manipulations were done at room temperature (20 ± 1 °C) (Porta-de-la-Riva *et al*., 2012).

3.2. Microbial strains and culture conditions

Stocks of *E. coli* OP50 were kept at -80 °C. Before each experiment the frozen aliquots were thawed on ice and then 0.1 ml was streaked out on Luria-Bertani (LB) agar plate (10 g/L sodium chloride, 5 g/L yeast extract; 10 g/L tryptone; 16 g/L agar) followed by incubation at 37 °C overnight. Thereafter, a single colony of the bacterium was inoculated into 5 ml LB broth and further incubated at 37 °C. After a 24 h cultivation cycle, a fresh bacterial culture was spotted on NGM agar followed by incubation at 37 °C overnight. *Candida albicans* SC5314 reference strain used in this study was acquired from the University of the Free State Yeast Culture Collection. The yeast strain was preserved on yeast extract-peptone-dextrose (YPD) agar (5 g/L peptone, 3 g/L yeast extract, 10 g/L glucose, 16 g/L agar) at 30 °C. *Pseudomonas aeruginosa* PAO1 reference strain used in this study was provided by Professor Hancock from the Department of Microbiology and Immunology at the University of British Columbia and maintained in LB broth at 37 °C.

3.3. Monomicrobial infection model

A previously described *C. elegans* liquid medium killing assay protocol by Breger and co-workers (2007) was modified for the establishment of monomicrobial infection by *C. albicans* and *P. aeruginosa* in *C. elegans* using a liquid medium pathogenesis assay. Freshly grown *C. albicans* cells were inoculated into 5 ml of YPD broth then incubated at 30 °C overnight, while *P. aeruginosa* cells were inoculated into 5 ml of LB broth medium followed by incubation at 37 °C overnight. Cell densities were adjusted to final cell concentration of approximately $l0^6$

cells/ml. An aliquot of 0.1 ml of the yeast and bacterial suspension was evenly spread onto a 10 mm square lawn on a large (100 mm x 15 mm) brain-heart infusion agar (BHI, Sigma-Aldrich) (7.8 g/L brain extract, 9.7 g/L heart extract, 2.5 g/L disodium phosphate, 2.0 g/L dextrose, 15 g/L agar) plate separately. Thereafter, yeast plates were incubated at 30 °C, while bacterial plates were incubated at 37 °C overnight.

Synchronized L4 *C. elegans* nematodes grown at 15 °C, as previously described in section 3.1, were washed from two large plates containing *E. coli* OP50 with a sterile M9 buffer (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, 0.25 g/L $MgSO_4.7H_2O$). Bacterial food source, which may skew the infection process, was removed from the samples via sucrose floatation prior to nematode collection (Jenkins, 1964). Briefly, using a 15 ml conical centrifuge tube, equal volumes of the washed nematodes preparation was mixed with a 60% sucrose solution to yield an approximate sucrose concentration of 30%, and a final fluid volume of 7 to 10 ml. The samples were kept on ice to inhibit movement of the nematodes, following which the samples were centrifuged in a cold centrifuge for 5 min at 6000 *g*. Floating nematodes were aspirated using a transfer pipette and expelled into a clean conical centrifuge tube. The harvested nematodes were washed again with distilled water and centrifuged at 6000 *g.* Following which 400 to 500 washed nematodes were placed on either the centre of *C. albicans* lawn or *P. aeruginosa* lawn grown on BHI agar. The BHI agar plates with infected nematodes and control nematodes, feeding on *E. coli* OP50, were incubated at 25 °C for 4 h. After incubation, nematodes were carefully washed off the BHI agar plates with 6 ml of sterile M9 buffer and transferred to a 15 ml conical tube and thoroughly washed with M9 buffer four times. Any microbial contaminants, which may confound the infection process, were again removed via sucrose floatation (Jenkins, 1964). About 60 to 70 nematodes were transferred into 2 ml of liquid medium containing 80% M9 buffer, 20% BHI and 90 µg/ml kanamycin in a single well of a six-well tissue culture plate (Greiner Cellstar, Sigma). Nematodes were monitored daily by scoring them as either alive, dead or dead with hyphal formation. If nematodes did not show any movement in response to picking mechanical stimulation, they were considered dead.

3.4. Polymicrobial infection model

The methodology used for the *C. elegans-P. aeruginosa-C. albicans* liquid medium assay was based on the previously described *C. elegans* liquid medium killing assay protocol by Breger and co-workers (2007). A similar process was followed as for monomicrobial infections (section 3.3), with the exception that approximately 400 to 500 washed nematodes were firstly placed on the centre of *C. albicans* lawns growing on BHI agar then incubated at 25 °C for 2 h. After the incubation period, nematodes were carefully washed off the BHI agar plates with

6 ml of sterile M9 buffer and transferred to 15 ml conical tube. Nematodes were washed four times with M9 buffer. Any microbial contaminants, which may confound the infection process, was removed via sucrose floatation during the washing step (Jenkins, 1964). Thereafter, washed nematodes were added to the centre of the *P. aeruginosa* lawns then incubated at 25 °C for further 2 h. After the incubation period, nematodes were carefully washed off the BHI agar plates with 6 ml of sterile M9 buffer and transferred to 15 ml conical tube. Importantly great care was taken to minimize the transfer of microbes into the conical tube. Nematodes were thoroughly washed with M9 buffer four times. About 60 to 70 nematodes were transferred into 2 ml of liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) in a single well of a six-well tissue culture plate. Nematodes were monitored daily by scoring them as either alive, dead or dead with hyphae piercing the cuticle. If nematodes did not show any movement in response to picking mechanical stimulation, they were considered dead.

3.5. Egg-in-worm assay

The egg-in-worm (EIW) assay of the infected nematodes were carried out according to Gardner *et al*. (2013). Lawns (10 mm) of either or both *C. albicans* and *P. aeruginosa* were prepared separately by plating 0.1 ml of freshly overnight culture of the yeast or bacterial strains on a large BHI agar plate, then incubated at 37 °C for 24 h. Synchronized L4 *C. elegans* nematodes, growing on two large NGM agar plates seeded with *E. coli* OP50 were washed off carefully with sterile M9 buffer. About 15 - 20 L4 *C. elegans* were picked and placed onto the centre of a lawn of either or both bacteria and yeast. Plates were then incubated for 40 h at 15 °C. A 20% bleach solution was prepared mixing a commercial bleach (6.0% sodium hypochlorite) with the appropriate volume of distilled water. Thereafter, 10 µl drops of bleach solution were added to ten distinct locations on a plastic petri dish lid. Using a pipette, one nematode was transferred into each bleach drop. The cuticle of the nematode was allowed to dissolve for approximately 10 min or until the nematode burst open, expelling the eggs. The number of retained eggs was quantified by counting while viewing under Olympus stereo dissecting microscope.

3.6. Influence of infection on nematode fatty acid composition

3.6.1. Extraction of fatty acids

Synchronized L4 *C. elegans* nematodes, infected with either or both *C. albicans* and *P. aeruginosa* were carefully harvested and washed three times with sterile M9 buffer.

Subsequently, the nematodes were transferred into six-well tissue culture plates containing 10 ml liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) and were incubated at 25 °C for 24 h. Following incubation, nematodes were washed three times by centrifugation at 4000 *g* for 2 min, the supernatant gently aspirated and the pellet containing the nematodes pulverized with a mortar and pestle to break open the nematodes for the release of their intracellular components. The total lipids of the nematodes were extracted overnight using 4 - 10 ml chloroform/methanol (2:1 v/v) solvent system (Folch *et al.*, 1957). Thereafter, the extract was filtered, and the supernatant (lipid containing phase) of the filtrate obtained was dried under nitrogen and stored at -80 °C prior to analysis using gas chromatography (GC).

3.6.2. Gas chromatography analysis

Fatty acids were esterified to form methyl esters (FAMEs) using 0.5 N NaOH and 14% boron trifluoride in methanol (Slover and Lanza, 1979; Hur *et al*., 2004; Diaz *et al*., 2005). Fatty acid methyl esters were quantified using a Varian 430 flame ionization gas chromatography (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses). All the analyses were performed using an initial isothermic period (40 °C for 2 min). Thereafter, the temperature was increased at a rate of 4 °C/min to 230 °C followed by an isothermic period of 230 °C for 10 min. The FAMEs were then dissolved in *n*-hexane and 1 µl was injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at a constant temperature of 250 °C. The hydrogen, at 45 psi, served as the carrier gas, while nitrogen served as the make-up gas. Finally, the chromatograms were recorded using the Galaxy Chromatography Software. The FAME samples were identified by comparing the retention times of authentic standards (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich). All other reagents and solvents that were used were of analytical grade and obtained from Merck Chemicals. The unsaturation index was calculated by characterizing each FA in a group based on the double bond number and multiplied each group by its double bond number. The unsaturation indixes of the extracted lipids of the control and supplemented was calculated as follows: Unsaturation Index = 1×[% monoenoic fatty acids] + 2×[% dienoic fatty acids] + 3×[% trienoic fatty acids] + 4×[% tetraenoic fatty acids] + 5×[% pentaenoic fatty acids] (Thibane *et al*., 2012)

3.7. Influence of infection on gene expression

3.7.1. Total RNA extraction

Synchronized L4 *C. elegans* nematodes, infected with either or both *C. albicans* and *P. aeruginosa,* were carefully harvested and washed three times with sterile M9 buffer. Subsequently, the nematodes were transferred into six-well tissue culture plates containing 10 ml liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) and were incubated at 25 °C for 24 h. Following incubation, nematodes were washed by centrifugation at 4000 *g* for 2 min, the supernatant gently aspirated from mono- and polymicrobial pellet and 2 ml RNA*later* (Invitrogen) added to each sample to combat degradation of RNA. This was done in triplicate for both mono- and polymicrobial infections. Samples were frozen at -80 °C until RNA extraction.

Samples were thawed on ice, centrifuged at 4000 *g* for 2 min to collect cells and RNA*later* was aspirated. The nematode pellet was resuspended in 600 µl of lysis buffer (Zymo Research), supplemented with 1 volume of glass beads (diameter 0.5 mm) and mechanically homogenized twice for 15 min using a Disruptor Genie Analog Cell Disruptor. Total RNA was extracted from samples using Quick-RNA MiniPrep kit (Zymo Research), including removal of the genomic DNA by DNase digestion, according to manufacturer's instructions. The RNA samples were evaluated using the Thermo Scientific NanoDrop ND-1000 Ultraviolet Visible Spectrophotometer to determine total RNA concentration in each sample.

3.7.2. Analyses of differential expression with nCounter®

Total RNA extracted from the different treatment conditions were analysed with the NanoString nCounter® analysis system (Geiss *et al*., 2008) at the University of the Witwatersrand, Department of Internal Medicine, using a gene expression TagSet that targets 123 *C. albicans* genes [including three housekeeping genes, *ACT1*, *LSC2* and *THD3* (Nailis *et al*., 2006)] and 60 *C. elegans* genes [including three housekeeping genes, *rps-2*, *rps-4* and *rps-23* (Tao *et al*., 2020)]. The full list of genes with functions and the sources of the 183-genes can be found in Supplementary Table S1. Analyses of differential expression was performed using nCounter® with Elements™ XT Reagents according to manufacturer's specifications. A multiplexed probe library (nCounter® elements CodeSet) was designed with two sequence-specific probes for genes of interest. Probes were mixed with approximately 100 ng of purified total RNA and allowed to hybridize (20 h, 67 °C). Samples were loaded on an nCounter® SPRINTTM Cartridge and processed with an nCounter® SPRINT Profiler (NanoString Technologies, USA) to quantify the transcripts. The nCounter raw expression data file (RCC)

obtained was uploaded into the nSolver Analysis Software 4.0 for review of quality control metrics. For this chapter, only data pertaining to *C. elegans* were used. The data was grouped between the experiments and control, and their expression ratio was determined. Genes with a fold change of ≥ 1.5 or ≤ -1.5 were identified from the gene list in each of the experimental conditions, and only the significantly differentially expressed genes ($P \leq 0.05$) were considered.

3.8. Statistical analyses

Each *C. elegans* pathogenesis assay was repeated in triplicate on separate occasions, and we calculated the mean and standard deviation. *C*. *elegans* survival was assessed using the Kaplan-Meier method and differences were determined with the log-rank test using OASIS 2 with statistical analyses performed using two-way ANOVA with Bonferroni correction (Han *et al*., 2016). All the other experiments were performed in triplicate and the average and standard deviation calculated. The student *t*-test was carried out to determine statistically significant differences between data sets. A *P*-value of ≤ 0.05 was considered significant.

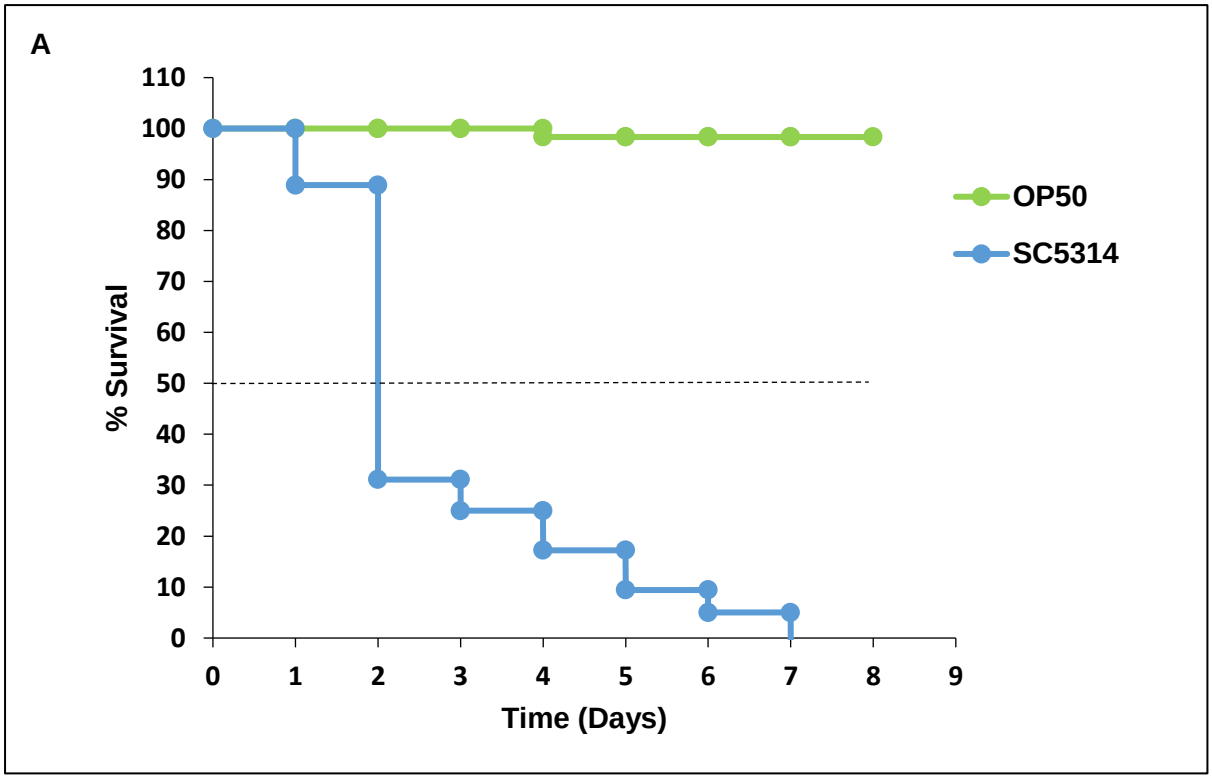
4. Results and Discussions

4.1. *Candida albicans* infection decreases nematode survival

Caenorhabditis elegans is a convenient biological model to study monomicrobial pathogenesis (Couillault and Ewbank, 2002; Balla and Troemel, 2013). The simple body structure and transparent nature of the nematode provides a simplified *in vivo* model, capable of mimicking the physiological and pathogenic mechanisms that takes place during infection (Brenner, 1974). Therefore, in this present study, we firstly used this nematode as an infection model to study *C. albicans* pathogenesis. After infecting *C. elegans* with *C. albicans* SC5314, we characterized the nematodes as either live or dead. Live nematodes maintained a sinusoidal shape and were motile while pumping their pharyngeal muscles. However, we saw that the dead infected nematodes were bloated with *C. albicans* cells and appeared rod shaped or straightened and rigid, with stretched and lengthened body. In addition, there was lack of mobility in dead infected nematodes. We also discovered that the survival of *C. albicans* SC5314 infected nematodes were significantly reduced compared to control nematodes fed with *E. coli* OP50 ($P < 0.001$) (Figure 2.1A). The virulence of *C. albicans* SC5314 towards *C. elegans* was visually evident within the first 2 days of infection, when *C. albicans* SC5314 killed 50% of the nematodes (Figure 2.1A). This rapid initial decline in nematode survival was followed by a second phase, in which nematodes died more slowly, taking 7 days for *C.*

albicans SC5314 to kill 100% of the nematodes (Figure 2.1A). Interestingly, in our assay, every *C. albicans* infected nematode that was killed during the first 2 days had visible hyphae piercing the cuticle (Figure 2.1B-C). Thereafter, we never observed any visible hyphae piercing the cuticle of infected nematodes until all nematodes were dead. This is similar to the results observed by Pukkila-Worley and co-workers (2009), who also found that more than half of the nematodes infected with *C. albicans* SC5314 died within 48 h and the dead nematodes showed hyphae piercing the cuticle. They also observed a second phase in which the nematodes died slower than the initial phase. In these latter nematodes, no hyphae were observed in either the intestine or piercing through the infected nematodes cuticle.

According to literature, *C. albicans* yeast cells have the ability to develop filaments that are usually differentiated to hyphae (long continuous germ tubes separated by true septin rings) or pseudohyphae (chains of distinct cells that fail to separate) (Lo *et al*., 1997; Braun and Johnson, 1997; Saville *et al*., 2003; Sudbery *et al*., 2004; Kadosh and Mundodi, 2020). Filamentation is an important feature of mammalian infection by *C. albicans*, where they are used to invade host tissues and exert mechanical force on cells and tissues (Lo *et al*., 1997; Braun and Johnson, 1997; Saville *et al*., 2003). Interestingly non-filamentous mutants of *C. albicans* have highly attenuated virulence (Lo *et al*., 1997; Mayer *et al*., 2013; Kadosh and Mundodi, 2020). Previous studies showed that *C. albicans* could vigorously form invasive hyphae in the *C. elegans* intestines, and that these hyphae could penetrate the nematode cuticle (Breger *et al*., 2007; Pukkila-Worley *et al*., 2009). However, mutants that cannot form hyphae seemed to be avirulent, implying that hyphal morphogenesis might be one of the mechanisms of nematode killing (Breger *et al*., 2007; Pukkila-Worley *et al*., 2009). It is also interesting that these assays are conducted at a temperature that does not normally induce hyphal formation (25 °C). Thus, studies of *C. albicans* morphogenesis in *C. elegans* also enable the analysis of hyphal growth triggers that are independent of temperature. We can speculate that environmental factors within the nematode gastrointestinal tract, which may have mammalian counterparts, may induce hyphal development in *C. albicans*. This aspect will be further studied in Chapter 4.



Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
***E. coli* OP50**	6.94	0.03	-	1.000
***C. albicans* SC5314**	2.79	0.12	2.00	< 0.001*

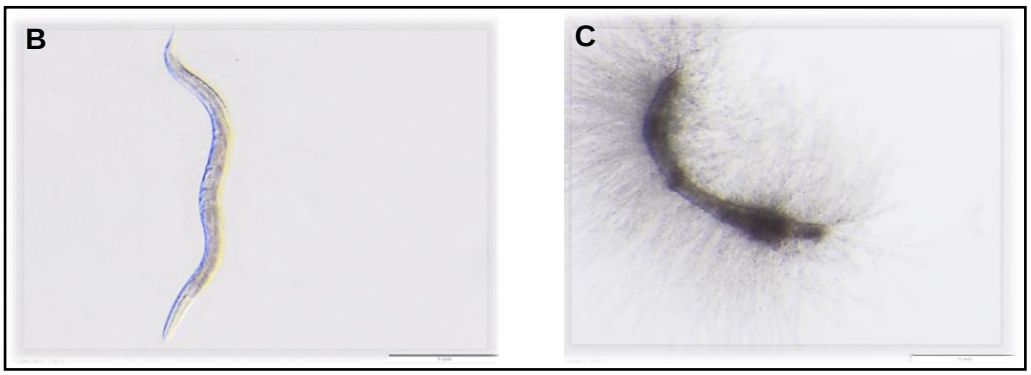
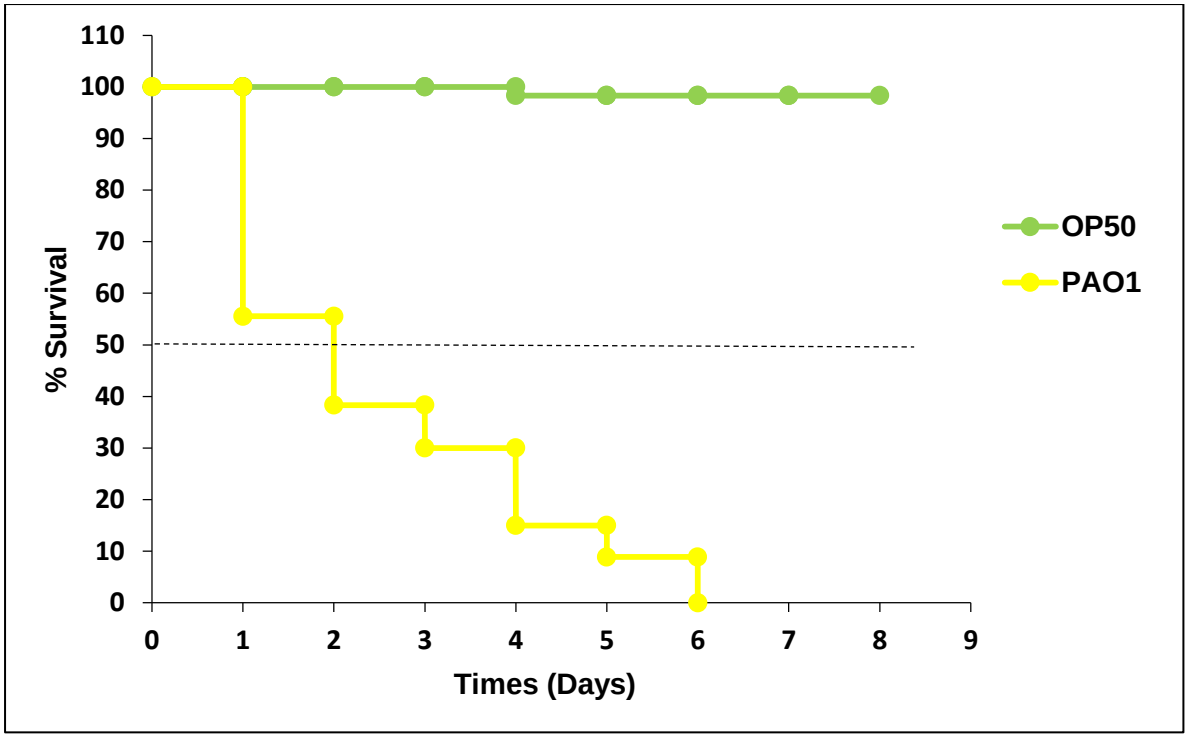


Figure 2.1. (A) *Candida albicans* killing larva 4 (L4) *Caenorhabditis elegans* larvae. The survival of nematodes was significantly reduced when exposed to *C. albicans* SC5314 compared to *Escherichia coli* OP50 control ($P \leq 0.05$). *C. albicans* SC5314 killed 100% of the

nematodes within 7 days. Dotted line represents 50% killing of the L4 larvae. The table represents median lifespan with standard error (S. E.) along with days to reach 50% mortality. Bonferroni *P*-values are included for the Log-rank test for overall differences in survival. Asterisk (*) indicate a significant difference from *E. coli* OP50. (B) Light micrograph of uninfected control nematode. (C) Light micrograph of nematode infected with *C. albicans* SC5314. Note presence of hyphae piercing the cuticle of a dead nematode within 2 days of infection.

4.2. *Pseudomonas aeruginosa* infection decreases nematode survival

Globally, bacterial infections have been predominantly associated with significant morbidity and mortality. This global burden could be overcome by understanding the mechanisms of both microbial virulence and host immune response. Previous studies showed that the nematode could be successfully used as an infection host used for numerous studies of reference strain *P. aeruginosa* PAO1 (Darby *et al*., 1999; Gallagher and Manoil, 2001). In this study, the survival of *P. aeruginosa* PAO1 infected nematodes was significantly reduced compared to uninfected nematodes ($P < 0.001$) (Figure 2.2). Pathogenicity was visually evident after 2 days of infection when *P. aeruginosa* killed 50% of the nematodes, with 100% mortality within 6 days. Previous studies by Kirienko and co-workers (2013) showed that in liquid medium killing assay, *P. aeruginosa* produces pyoverdine, which induces a hypoxic response, leading to the death of the nematode.

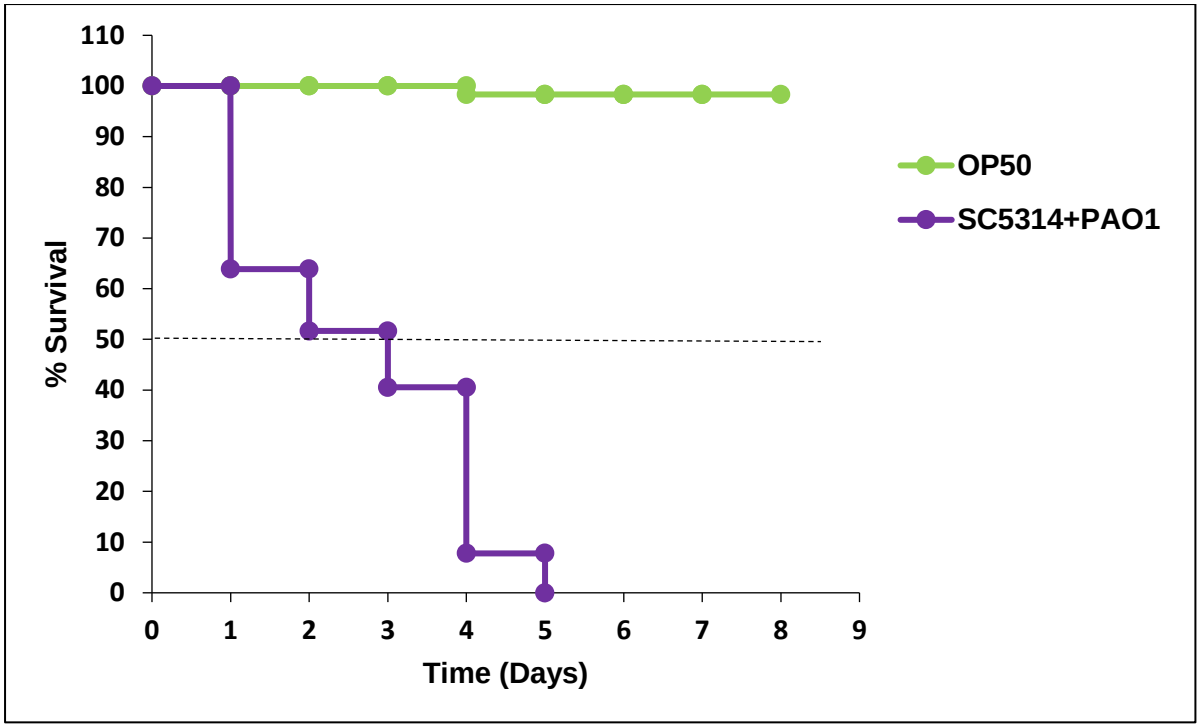


Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
***E. coli* OP50**	6.94	0.03	-	1.000
***P. aeruginosa* PAO1**	2.48	0.12	2.00	< 0.001*

Figure 2.2. Survival curves of larva 4 (L4) *Caenorhabditis elegans* nematodes exposed to *Pseudomonas aeruginosa*. The survival of nematodes were significantly reduced when exposed to *P. aeruginosa* PAO1 ($P \leq 0.05$). *P. aeruginosa* PAO1 killed 100% of the nematodes within 6 days. Dotted line represents 50% killing of the L4 larvae. The table represents median lifespan with standard error (S. E.) along with days to reach 50% mortality. Bonferroni *P*-values are included for the Log-rank test for overall differences in survival. Asterisk (*) indicate a significant difference from control nematodes (*E. coli* OP50).

4.3. *Candida albicans-Pseudomonas aeruginosa* co-infection increases virulence in *C. elegans* infection model

Bacterial-fungal interactions can display a diverse spectrum of effects, which may not be identical *in vivo* and *in vitro* (Fourie and Pohl, 2019). For instance, the interactions between *C. albicans* and *P. aeruginosa* observed *in vitro* are mostly antagonistic, but the interaction in the animal hosts, such as rat and zebrafish, displays synergistic effects on virulence, thus resulting in higher mortality (Roux *et al*., 2009; Bergeron *et al*., 2017; Nogueira *et al*., 2019). These differential effects may be explained by the host environment and the increased inflammatory response associated with cytokine profiles that are absent *in vitro* and also differ from single pathogen infections (Roux *et al*., 2009; Bergeron *et al*., 2017; Nogueira *et al*., 2019). Therefore, in this study, we used *C. elegans* to decipher the effect of polymicrobial infections of *C. albicans* and *P. aeruginosa*. As depicted in figure 2.3, we observed a significant reduction in the survival of *C. elegans* when infected with both *C. albicans* SC5314 and *P. aeruginosa* PAO1 compared to *E. coli* OP50 control strain ($P < 0.001$). Moreover, although *C. albicans* and *P. aeruginosa* can independently kill the nematodes, polymicrobial infection involving these pathogens significantly reduced the survival of the nematodes compared with monomicrobial infection, killing 100% of the nematodes within 5 days. We therefore can conclude that polymicrobial infections are more virulent than monomicrobial infections, given that in monomicrobial infections, *P. aeruginosa* killed 100% of the nematodes within 6 days (Figure 2.2), while *C. albicans* killed 100% of the nematodes within 7 days (Figure 2.1A). This suggests that the interaction between *C. albicans* and *P. aeruginosa* in this model displays synergistic effects on the virulence, thus resulting in higher mortality. It would be interesting to investigate the microbial burden to address the issue whether the observed virulence of both *C. albicans* and *P. aeruginosa* could be correlated to higher microbial burdens in *C. elegans*.



Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
***E. coli* OP50**	6.94	0.03	-	1.000
***C. albicans* SC5314 and *P. aeruginosa* PAO1**	2.64	0.11	3.00	< 0.001*

Figure 2.3. Effect of *Candida albicans* and *Pseudomonas aeruginosa* co-infection on Larva 4 (L4) nematodes over time. Co-infection killed 50% of the L4 larvae after 3 days, while 100% of nematodes were killed within 5 days. Dotted line represents 50% killing of the L4 larvae. The table represents median lifespan with standard error (S. E.) along with days to reach 50% mortality. Bonferroni *P*-values are included for the Log-rank test for overall differences in survival. Asterisk (*) indicate a significant difference from control (*E. coli* OP50).

Importantly, we did not observe any hyphal formation by *C. albicans* SC5314 throughout the polymicrobial infection. It is conceivable that *P. aeruginosa* may play a role in the observed hyphal inhibition of *C. albicans*. This is especially interesting since the polymicrobial infection was not less virulent (as would be expected if *C. albicans* hyphal formation was important in this context). Thus, we can speculate that the killing of the nematodes infected with both pathogens was not due to hyphal formation but maybe because of other virulence factors of both *C. albicans* SC5314 and *P. aeruginosa* PAO1. Similar results were also seen in the study done by Peleg and colleagues (2008), when nematodes were infected sequentially with *C. albicans* followed by infection with *P. aeruginosa*, and filamentation by *C. albicans* was significantly inhibited.

The relevance of this observation can also be seen in other infection models. Similarly, in an immunocompetent murine burn model, pre-infection with *P. aeruginosa* increased the damage caused by *C. albicans* (Neely *et al*., 1986). A sublethal *P. aeruginosa* injection followed by a sublethal fungal injection yielded high mortality. Roux and colleagues (2009) further used immunocompetent rats to evaluate the relationship between *C. albicans* colonisation and the existence of *P. aeruginosa* pneumonia in lungs. They found that *C. albicans* enhanced infection of *P. aeruginosa* by impairing macrophage function, causing a significant increase in the occurrence of *P. aeruginosa* pneumonia (Roux *et al*., 2009). Moreover, these authors hardly observed any hyphal form of *C. albicans* in the rat lungs (Roux *et al*., 2009; Tan *et al*., 2016). Furthermore, in a transparent juvenile zebrafish model of mucosal lung infection, *C. albicans* and *P. aeruginosa* co-infection of the mucosa led to synergistic virulence and enhanced mortality (Bergeron *et al*., 2017). The authors also discovered that the co-infection mortality was associated with higher *C. albicans* burden and invasive pathogenesis, which serve as strong predictive factors for mortality. By contrast, numerous studies of *C. albicans-P. aeruginosa* co-infections in murine and *C. elegans* models show that the interaction of these two species can negatively regulate overall virulence in the context of co-infection (Peleg *et al*., 2008, 2010; Lopez-Medina *et al*., 2015). These varying results suggest that the outcome of *C. albicans-P. aeruginosa* co-infections is not easily predicted from *in vitro* antagonism (Xu *et al*., 2014a; Nash *et al*., 2015; Schlecht *et al*., 2015; Pendleton *et al*., 2017). Despite the fact that the mortality rates in some *in vivo* models, such as the mouse and zebrafish were elevated, possibly due to exacerbation of inflammatory responses, there are still controversies concerning the differential outcomes in various animal models (Neely *et al*., 1986; Méar *et al*., 2013; Leclair and Hogan, 2010; Chotirmall *et al*., 2010; Bergeron *et al*., 2017). For majority of the bacterial and fungal interactions outlined above, there is minimal to no data available concerning the interplay with the host and the immune system response towards these polymicrobial infections in comparison to the respective monomicrobial infections.

We can further speculate in this study that the effects of *C. albicans* and *P. aeruginosa* interaction on *C. elegans* may not only depend on the virulence factors of pathogenic strains but also on the immune status of the host. Importantly, since *C. elegans sek-1* mutant strain lacks the *SEK-1* gene that encodes a conserved mitogen-activated protein kinase (MAPKK) involved in the innate immune response, these nematodes are immunocompromised, allowing them to become infected with various pathogens (Kim *et al*., 2002). Noteworthy, perturbations of the microorganisms and a weakened host immune system are conditions that can facilitate the transition of opportunistic microorganisms from a commensal state to a pathogenic state, thus resulting in the initiation of infection. It is known that the immune response, including the degree of inflammation, can exert a major effect on factors affecting the pathogenicity and virulence of the individual pathogens involved, and may thus affect the overall result of the bacterial-fungal interaction. However, the immune response is not always effective. Fungi have developed several mechanisms and strategies to escape the attack of the immune system (Nogueira *et al*., 2019). The escape mechanisms essentially include shielding of pathogen-associated molecular patterns (PAMPs) through the cell wall or capsule, and the formation of biofilms, titan cells, asteroid bodies, or dimorphism (yeast to hyphal transition) (Nogueira *et al*., 2019). For example, certain virulence factors of *C. albicans* are exclusively expressed at the hyphal stage, and hyphal cells induce low cytokine production compared to yeast cells (Mukaremera *et al*., 2017). Hyphal structures are also important to evade phagocytosis and escape from the immune cells (Hernandez-Chavez *et al.,* 2017). Bacteria have also developed mechanisms to hide or escape from the immune system (Nogueira *et al*., 2019). Some of these mechanisms are similar to those used by fungi. Biofilm formation is also an important feature used by bacteria to evade the immune response (Cheung *et al*., 2021). Other factors include the secretion of proteins, quorum sensing regulation, production of antigenic exotoxins, pore-forming toxins, and capsular polysaccharides (Nogueira *et al*., 2019; Cheung *et al*., 2021). Therefore, escape from the immune system may lead to persistent and chronic infection, bearing the risk of potentially life-threatening reactivation occurring particularly in severely immunocompromised individuals. Taken together, it is clear that yeast-bacterial polymicrobial interactions can either display synergistic or antagonistic interactions, depending on either the *in vitro* or *in vivo* studies. In addition, it is possible for co-infections to be more virulent compared to single infections. Thus, from our observations we can speculate that *C. albicans* and *P. aeruginosa* interactions indicate complex, synergistic yeast-bacterial interactions in *C. elegans* infection model and that more studies are needed to assess the potential importance of these pathogens in the host setting.

4.4. Influence of infection on nematode egg retention

Caenorhabditis elegans encounters a variety of food sources, including pathogenic microorganisms that are potentially harmful. Thus, when exposed to harmful agents in the environment, *C. elegans* retains eggs until the environment becomes more favourable (Chase and Koelle, 2004; Schafer, 2005; Gardner *et al*., 2013). In this assay, we evaluated the number of retained eggs within *C. elegans* after exposure to either or both *C. albicans* and *P. aeruginosa*. According to literature, in reproductively mature *C. elegans* hermaphrodites, fertilised eggs are retained within the uterus for several hours before being laid (Schafer, 2005). The normal number of eggs stored in the uterus during peak fecundity is between 5 and 10 as *C. elegans* is raised on their natural food source *E. coli* (Schafer, 2005). Importantly, the number of eggs in the uterus is a function of both the rate of egg production and the rate of egg laying (Schafer, 2005; Gardner *et al*., 2013). In our study, nematodes that were raised on *E. coli* OP50 fell into this range, with about 7 (±1.70) eggs retained (Figure 2.4). When *C. elegans* were exposed to the different pathogens, we observed increases in egg retention (Figure 2.4), with more eggs retained in the presence of *C. albicans* SC5314 (10.8±2.35; P = 0.0013), *P. aeruginosa* PAO1 (12.2±3.55; P = 0.0001) and most retained in the presence of both *C. albicans* and *P. aeruginosa* (17.5±3.65; P = 0.0001), compared to *E. coli* OP50. The higher egg retention correlates with the increased virulence of the co-infection observed in the liquid killing assay (Figure 2.3). Similar results were also observed where more eggs were retained in the presence of *E. faecalis* pathogenic strains, compared to *E. coli* OP50 controls (Gardner *et al*., 2013).

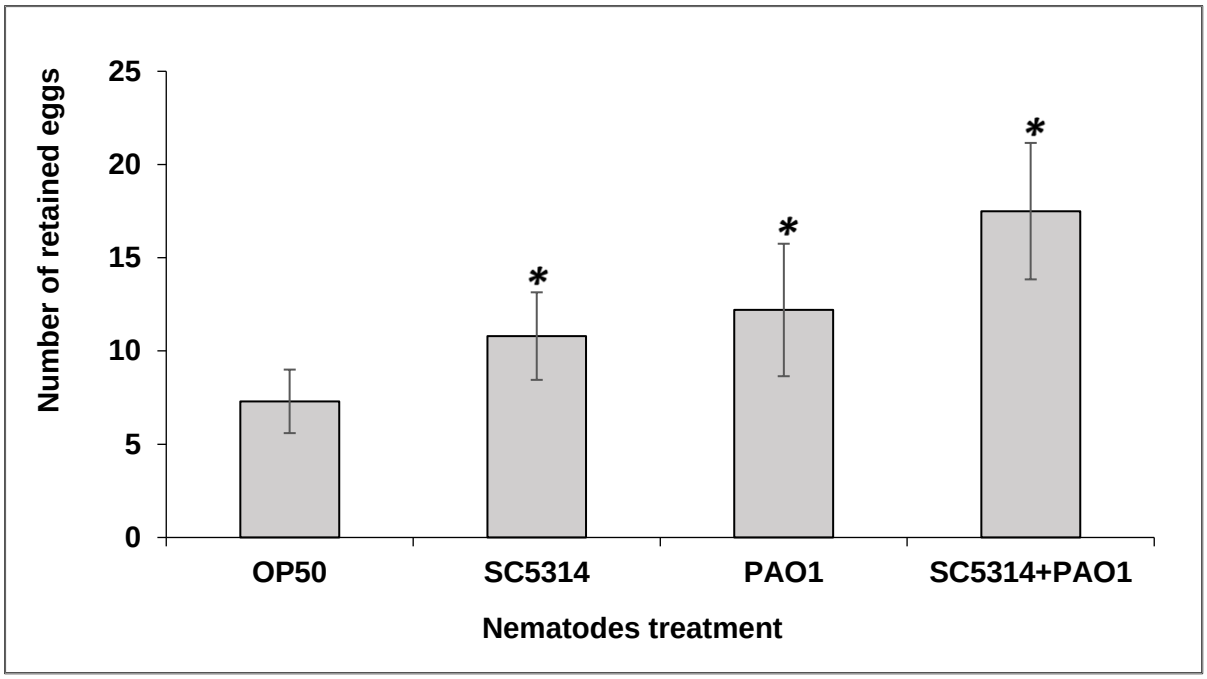


Figure 2.4. Egg-in-worm assay of *Caenorhabditis elegans* infected with *Candida albicans* and *Pseudomonas aeruginosa*. Nematodes exposed to *C. albicans* SC5314 and *P. aeruginosa* PAO1 showed an increased level of egg retention compared to nematodes fed the *Escherichia coli* OP50 control strain. Each experiment was performed on triplicate on different days on ten nematodes each. Asterisk (*) indicate $P < 0.05$ compared to control (Student's *t*-test).

4.5. Influence of infection on nematode fatty acid composition

Emerging lipidomic technologies offer exciting opportunities to us understanding the influence of lipids on lifespan. Due to the detailed characterization of metabolic pathways and ease of genetic manipulation, the nematode *C. elegans* has emerged as a powerful model organism for studying lipids (Mullaney and Ashrafi, 2009; Watts, 2009). Despite the observed differences in lipids storage and synthesize between nematodes and mammals, some lipid profiles associated with longevity are conserved, and *C. elegans* has revealed molecular mechanisms relating lipids to lifespan with a potential to be explored in mammals (Mullaney and Ashrafi, 2009). Although numerous studies have revealed the impact of dietary FA interventions at the level of gene regulation and transcription, there is minimum research on the changes in the FA metabolite profiles in *C. elegans* after co-infection. We, therefore, applied our platform to uncover the changes in the FA profiles of *C. elegans* after infection with *C. albicans* and *P. aeruginosa*. Firstly, from the results of the quantitative analyses of FAMEs derived from the lipids of *C. elegans* shown in Table 2.1, we identified 23 different FAs from the *C. elegans* fed

on control *E. coli* OP50. The identified FAs ranged from 12 to 22 carbons in length, and 35% were saturated, while 65% were unsaturated. The results we obtained followed previous reported results that state that *E. coli* OP50 as a bacterial food source of *C. elegans* contains saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs), including palmitoleic acid (16:1n-9) and vaccenic acid (18:1n-7), but not oleic acid (18:1n-9), in their membrane. As nematodes feed on bacteria, these FAs can be incorporated into the nematode lipid composition. In a recent study by Henry and colleagues (2016), about 28 different FAs were observed, with a length ranging from 12 to 22 carbons, and a majority of those consisting of 12 to 17 carbons, were saturated, with the exception of 16:1n-9 and *cis*-10-heptadecenoic acid (17:1). However, unlike with our current study, stearic acid (18:0) was the most predominant SFA, with an average of 6.5% of total FAs. Furthermore, Henry and colleagues (2016) obtained other saturated acids, such as lauric acid (12:0), tridecanoic acid (13:0), myristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (16:0), margaric acid (17:0), arachidic acid (20:0) and docosanoic acid (22:0). Interestingly, similarly to our results, Henry *et al*. (2016) showed that 65% of the identified FAs were unsaturated comprising 18 to 20 carbon atoms, and monounsaturated acids accounted for 35.6% of the total FAs. Moreover, they identified 16:1n-9, 18:1n-9 and gadoleic acid (20:1). The FA 18:1n-7 comprised close to 62% of MUFAs. Other FAs that were detected were linoleic acid (18:2n-6), eicosatrienoic acid (20:3n-3), dihomo-gamma-linolenic acid (20:3n-6), eicosapentaenoic acid (20:5n-3) gamma-linolenic acid (18:3n-6), alpha-linolenic acid (18:3n-3), and two forms of arachidonic acid (20:4n-6 and 20:4n-3) (Henry *et al*., 2016).

Table 2.1. The quantitative analyses of FAMEs derived from fatty acids of *Caenorhabidtis elegans* grown on *Escherichia coli* OP50.

Common name	**Formula**
Lauric acid	12:0
Myristic acid	14:0
Pentadecanoic acid	15:0
Palmitic acid	16:0
Palmitoleic acid	16:1n-9
Margaric acid	17:0
Stearic acid	18:0
Oleic acid	18:1n-9
Vaccenic acid	18:1n-7
Linoleic acid	18:2n-6
γ-Linolenic acid	18:3n-6
α-Linolenic acid	18:3n-3
Stearidonic acid	18:4n-3
Nonadecanoic acid	19:0
Arachidic acid	20:0
Eicosadienoic acid	20:2n-6
Eicosatrienoic acid	20:3n-6
Eicosatrienoic acid	20:3n-3
Arachidonic acid	20:4n-6
Eicosopentaenoic acid	20:5n-3
Erucic acid	22:1n-9
Docosadienoic acid	22:2n-6
Docosapentaenoic acid	22:5n-3

In order to explore the effect of infection on nematode FA metabolism with focus on the abundant major unsubstituted long chain fatty acids (LC-FAs), we exposed the nematodes to either or both *C. albicans* and *P. aeruginosa*. We discovered that the total lipids of control nematodes had a higher unsaturation index compared to infected nematodes (Figure 2.5A). Although this difference was not statistically significant in the case of nematodes infected with *C. albicans* ($P = 0.083$), nematodes infected with *P. aeruginosa* showed a significant decrease in unsaturation index ($P = 0.0058$), while the co-infection exhibited the highest decrease in unsaturation index ($P = 0.0006$) (Figure 2.5A). We further observed a high abundance of 20:5n-3 in control nematodes compared to *P. aeruginosa* and co-infected nematodes, which showed a significant decrease in 20:5n-3 (Figure 2.5B). Therefore, we can speculate that the observed low unsaturation index of the *P. aeruginosa* and co-infected nematodes is partially due to the decrease in relative percentage 20:5n-3 after infection.

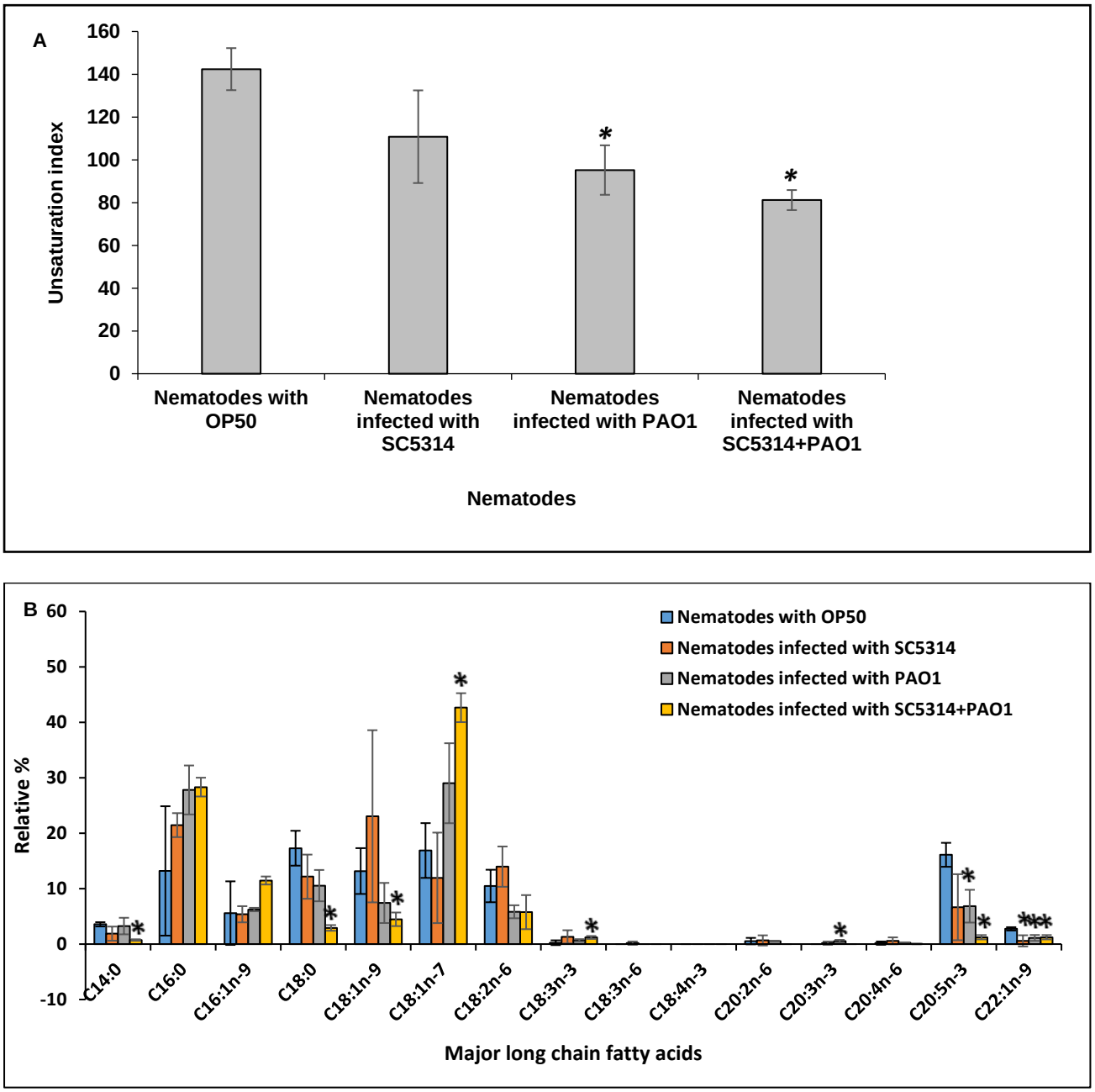


Figure 2.5. (A) Unsaturation index of *Caenorhabditis elegans* infected with *Candida albicans*, *Pseudomonas aeruginosa* and co-infection, compared to uninfected *C. elegans* on *Escherichia coli*. (B) Effect of *C. albicans* and *P. aeruginosa* on relative percentage of major long chain fatty acids as determined by gas chromatography analysis in *C. elegans*. Values represent the mean of three independent experiments, while error bars represent the standard deviations and asterisk (*) indicate a significant difference from control with *P*-values < 0.05

We also discovered that infection with *P. aeruginosa* PAO1 caused other changes in the relative percentage FAs of the nematodes (Figure 2.5B). There was a significant decrease in 20:3n-3 and 22:1n-9. Interestingly, Nandakumar and Tan (2008) reported a similar trend where nematodes infected with both *P. aeruginosa* PA14 and an avirulent mutant (PA14DgacA) showed an increase in relative percentage of 20:5n-3 and had more than twice the 18:1n-7 content compared to control (*E. coli* OP50-1) nematodes. The authors argued that the higher levels of 18:1n-7 in the infected nematode is most likely due to nutritional differences between *P. aeruginosa* and *E. coli* rather than differences in virulence. Similarly, in our study there was an increase in 18:1n-7, although this was not statistically significant compared to the control nematodes (Figure 2.5B). Interestingly, although infection with *C. albicans* did not cause significant changes in the relative percentage of any FA, co-infection with *P. aeruginosa* and *C. albicans* exacerbated the changes observed during *P. aeruginosa* monomicrobial infections (Figure 2.5B) and resulted in significant decreases in the relative percentage of 14:0, 18:0, 18:1n-9, 20:5n-3 and 22:1n-9 as well as a significant increase in 18:1n-7. These two results indicate that the changes may not be due to nutritional changes as speculated by Nandakumar and Tan (2008) but may be a pathogen specific response of the nematode. The continued use of lipidomics and genetic studies of lipid synthesis and signalling pathways in model organisms will undoubtedly reveal many new roles for lipids in the regulation of pathogenicity and deepen our understanding of the complex relationship between lipids and pathogenicity.

4.6. Differential gene expression

To better understand the observed changes in virulence and FA composition in infected nematodes, we further investigated the influence of infections on expression of 57 selected *C. elegans* candidate genes related to lipid metabolism as well as the nematode immune response (Supplementary Table S1). We discovered that the responses to bacterial and fungal pathogens are remarkably distinct. The genes that are up-regulated and down-regulated differed between the experimental conditions (Figure 2.6). Table in figure 2.7 depicts the different conditions tested with their respective up-regulated genes involved in lipid metabolism and immune response. Genes with a fold change of ≥ 1.5 or ≤ -1.5 were identified from the gene list in each of the experimental conditions, and only the significantly up-regulated genes ($P \leq 0.05$) were considered. Of the nine *C. elegans* genes known to be involved in the synthesis of the majority of 18- and 20-carbon PUFAs and MUFAs (Figure 2.6), only *fat-4* was up-regulated ($P \leq 0.05$) in the nematodes infected with either *P. aeruginosa* or combination of *C. albicans* and *P. aeruginosa* (Figure 2.7). According to Watts and Browse

(2002), *fat-4* functions to modify 20-carbon PUFAs to produce 20:4n-6 and 20:5n-3. In the previous section (Figure 2.5B), we observed a decrease in the relative percentage of 20:5n-3 in nematodes that were infected with either *P. aeruginosa* or co-infected. We can speculate that these results indicate a response, where decrease in LC-FA levels during infection could induce increased expression of corresponding biosynthetic genes. Similar results were observed in studies performed by Nandakumar and Tan (2008), where *fat-6*, *fat-2*, and *fat-3*, involved in the synthesis of FAs were up-regulated in response to infection. Moreover, in our study, of the five *elo* genes of unknown function, *elo-9* (encoding a putative very LC-FA elongase) was also significantly up-regulated ($P \leq 0.05$) during *P. aeruginosa* and polymicrobial infection conditions (Figure 2.7). This gene is possibly involved in stress responses and lifespan increase (Fajardo *et al*., 2019).

Research revealed that within the *C. elegans* genome, 86 full length cytochrome P450 (CYP) genes are present, but the exact biological functions of the vast majority of CYPs are largely unknown (Yadav *et al*., 2021). Thirty-eight of the CYPs may play a role in lipid metabolism, where they function in the synthesis of oxylipins (including eicosanoids), which are oxygenated FAs synthesized from precursor PUFAs (Zhang *et al*., 2013). Others may play roles in the immune response and detoxification (Mao *et al*., 2019). In this study, we observed an up-regulation of *cyp-14A2* ($P \leq 0.05$) in co-infected nematodes and up-regulation of *cyp-29A2* ($P \leq 0.05$) in both *P. aeruginosa* and co-infected nematodes (Figure 2.7). The gene, *cyp-29A2* is expressed in the intestine and plays a role in lipid storage and life span (Zhang *et al*., 2013). While *cyp-14A2* is involved in stress response and detoxification (Thomas, 2007). Therefore, we can speculate that infections can cause oxidative stress, which influenced expression of genes involved in stress response and life span described above. Further research is needed to determine if these CYP450s could affect susceptibility in infected *C. elegans.*

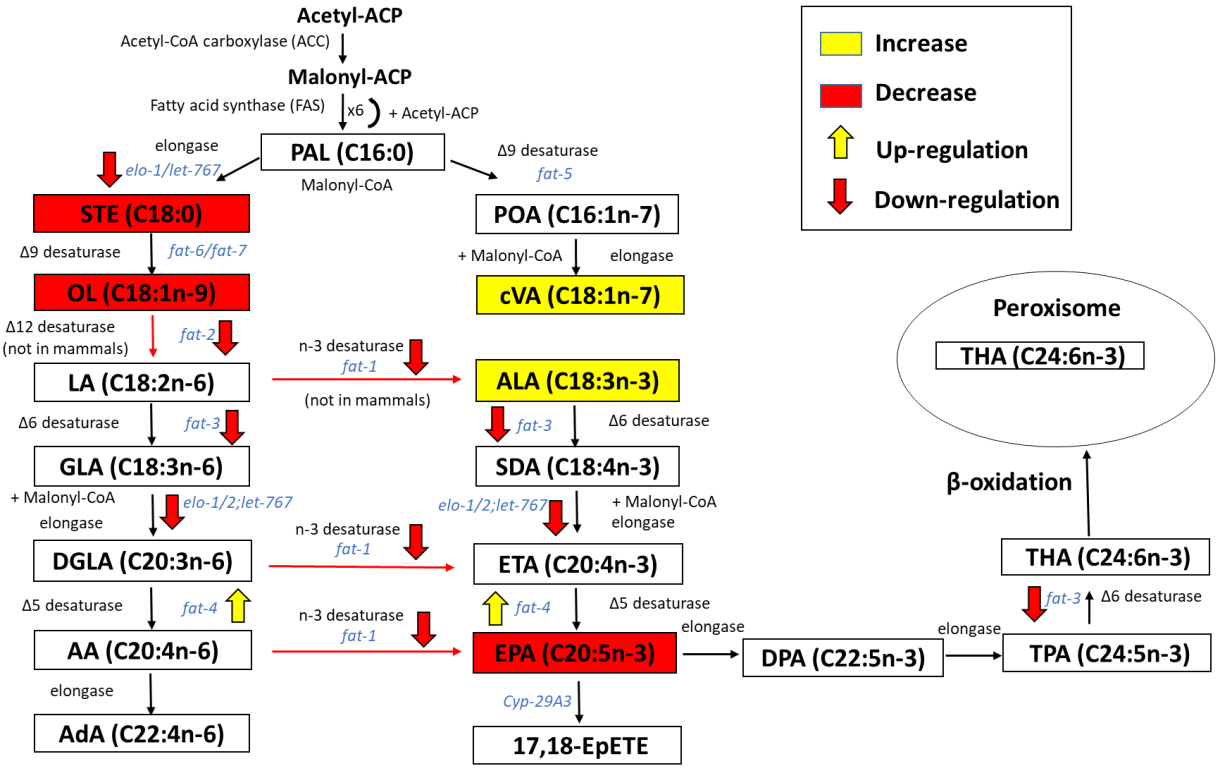
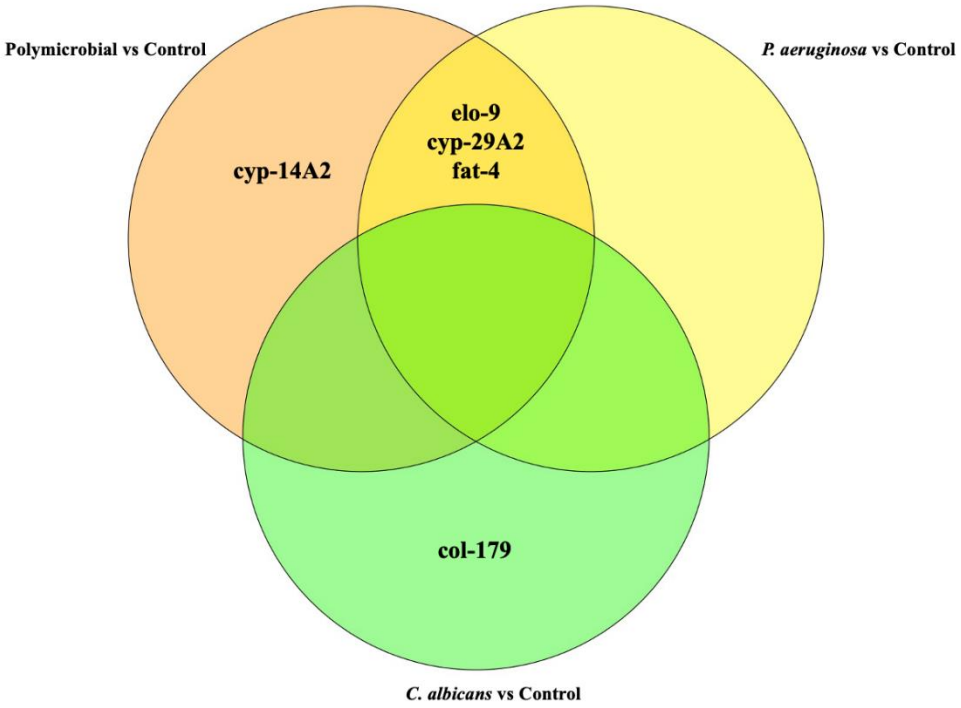


Figure 2.6. Effect of *Candida albicans* and *Pseudomonas aeruginosa* infection on *Caenorhabditis elegans* polyunsaturated fatty acids biosynthesis pathway. Yellow squares indicate significant increase, while red squares indicate significant decrease in relative percentage of fatty acids compared to control *Escherichia coli* OP50 nematodes. Yellow arrows indicate up-regulation, while red arrows indicate down-regulation in genes compared to control nematodes. Mammalian enzymes are drawn by solid black reaction arrows. Although this is a conserved pathway for both mammals and *C. elegans*, the nematode possesses Δ12 and omega-3 desaturase enzymes that are solid red arrows. Abbreviations: AA, arachidonic acid; ACC, acetyl-CoA carboxylase; ACP, acyl carrier proteins; Ada, docosatetraenoic acid; ALA, α-linolenic acid; cVA, *cis*-vaccenic acid; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; FAS, fatty acid synthase; GLA, γ-linolenic acid; LA, linolenic acid; OL, oleic acid; PAL, palmitic acid; POA, palmitoleic acid; SDA, stearidonic acid; STE, stearic acid; THA, tetracosahexaenoic acid (nisinic acid), TPA, tetracosapentaenoic acid; 17,18-EpETE, 17,18-epoxy eicosatetraenoic acid (Mokoena *et al*., 2020).



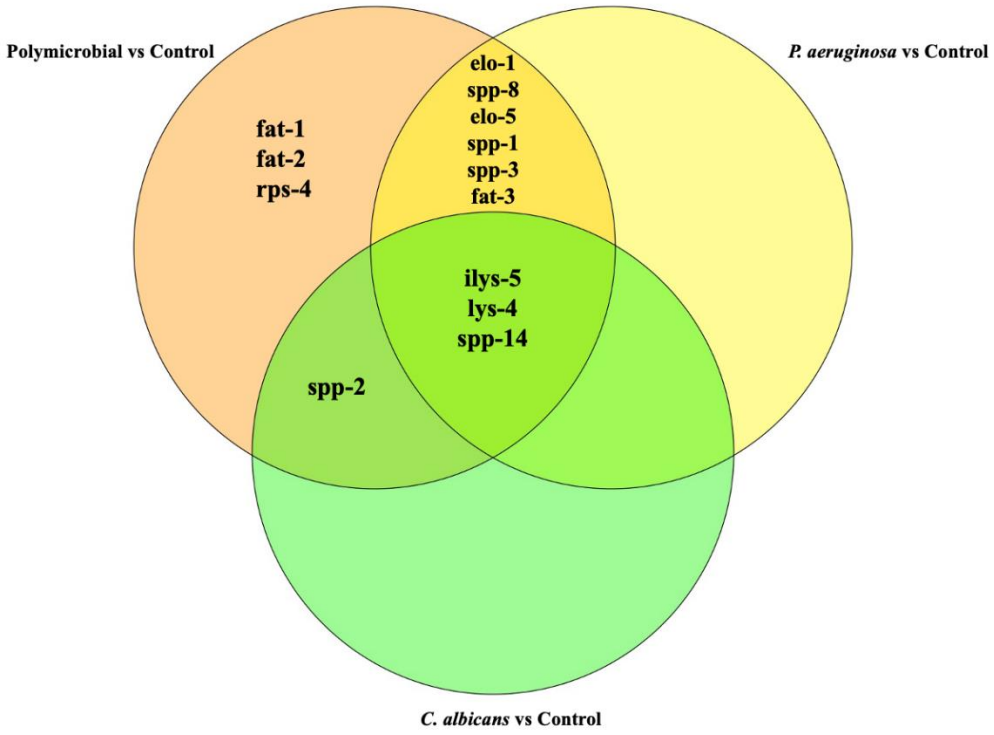
Conditions	Genes	Fold change	*P*-value	Lower 95% CI	Upper 95% CI
***Candida albicans* vs control**	*col-179*	1.67	0.0495	0.99	2.81
***Pseudomonas aeruginosa* vs control**	*cyp-29A2*	26.64	0.0254	1.40	508.22
	elo-9	20.07	0.0237	1.38	292.04
	fat-4	6.00	0.0226	1.03	34.93
Polymicrobial vs control	*cyp-14A2*	45.07	0.0008	16.04	126.68
	cyp-29A2	32.08	0.0374	1.36	754.46
	elo-9	32.61	0.0234	2.51	423.79
	fat-4	8.42	0.0012	4.10	17.26

Figure 2.7. Venn diagram depicting the overlap of up-regulated genes of *Caenorhabditis elegans* in response to *Candida albicans* and *Pseudomonas aeruginosa* infection compared to uninfected control nematodes. The table represents genes with a fold change of ≥ 1.5. *P*-values ≤ 0.05 indicate a significant difference from control, with lower and upper percentage confidence intervals (CI).

Table in figure 2.8 depicts the different conditions tested with their respective down-regulated genes involved in lipid metabolism and immune response. In co-infected nematodes, we observed a down-regulation of *fat-1* and *fat-2* ($P \leq 0.05$) (Figure 2.8). According to literature, *fat-1* encodes an omega-3 desaturase (Δ15 desaturase) that converts 18:2n-6 and γ-linolenic acid (18:3n-6) to α-linolenic acid (18:3n-3) and stearidonic acid (18:4n-3) (Spychalla *et al*., 1997), while *fat-2* encodes the Δ12 desaturase enzyme, which facilitates the biosynthesis of 18:2n-6 from its substrate, 18:1n-9 (Figure 2.6) (Zhou *et al*., 2011; Wang *et al*., 2013). We observed a decrease in relative percentage of PUFAs in co-infected nematodes (Figure 2.5B) which corresponds with the observed down-regulation in expression of *fat-1* and *fat-2* in co-infection (Figure 2.8). This may explain the observed increase in *cis*-vaccenic acid (18:1n-7) (Figure 2.5B), as the FA metabolism is rerouted along this branch of the pathway (Figure 2.6). In addition, as depicted in figure 2.8, in nematodes infected with either *P. aeruginosa* or co-infected, we saw a down-regulation of *elo-1* and *elo*-5*,* which encodes specific elongase enzymes that facilitate the elongation of both saturated and unsaturated FAs (Watts and Ristow, 2017). The gene encoding Δ6 desaturase involved in the biosynthesis of 18:3n-6 and 18:4n-3, *fat-3*, is also down-regulated in all infection containing *P. aeruginosa* (Figure 2.8). Studies performed by Nandakumar and Tan (2008) demonstrated that *fat-3* is expressed in the intestine, which is the site of *P. aeruginosa* infection, to protect *C. elegans* from pathogen-mediated death and to regulate the expression of immunity genes. According to Nandakumar and Tan (2008), loss of this gene resulted in nematodes that lacked 18:3n-6 and 18:4n-3, as well as all the 20-carbon PUFAs. Moreover, these nematodes were also significantly more susceptible to infection, suggesting that these PUFAs could be vital for infection response in *C. elegans* (Nandakumar and Tan, 2008).

Despite lacking adaptive immunity, *C. elegans* has an immune system that resembles the human innate immune system in several key respects and can trigger evolutionarily conserved immune signalling pathways (Mahajan-Miklos *et al*., 1999). Activation of these pathways induces the expression of defence genes such as those encoding lectins, lysozymes, lipases, and antimicrobial peptides, which then act directly or indirectly to combat invading microbes (Alper *et al*., 2007; Evans *et al*., 2008; Kong *et al*., 2016). For instance, the lysozyme (lys) family, *Ascaris suum* antibacterial factor (abf) family, saposin-like proteins (spp) family, and C-type lectins family are some examples of the nematode innate immunity effectors, which have been shown to play an important role in the general and induced immune responses to bacterial infection (Mochii *et al*., 1999; Couillault *et al*., 2004; Huffman *et al*., 2004; Dierking *et al*., 2016; Gravato-Nobre *et al*., 2016). Notably in the present work, expression levels of eight *spp* genes (*spp-1* to *spp-4*, *spp-8*, *spp-12*, *spp-14* and *spp-23*) were studied (Supplementary Table S1) and it was found that *spp-1*, *spp-3* and *spp-8* were down-regulated significantly

($P \leq 0.05$) in *C. elegans* in response to either *P. aeruginosa* or co-infection, while *spp-14* was down-regulated ($P \leq 0.05$) in all infection conditions (Figure 2.8). It is known that *P. aeruginosa* can down-regulate these immune effector genes, such as *spp-1*, *spp-4* and *spp-23* (Evans *et al*., 2008). It is also important to note that *C. elegans* has 16 lysozyme genes (Dierking *et al*., 2016). These are classified into two groups: the *lys*-genes, which are similar to those of protists, and the *ilys*-genes, which are similar to those of invertebrates (Schulenburg and Boehnisch, 2008; Gravato-Nobre *et al*., 2016). In this study, the expression levels of two *ilys*-genes (*ilys-2* and *ilys-5*) and five *lys*-genes (Supplementary Table S1) were determined and significant ($P \leq 0.05$) down-regulation of *ilys-5* in all infections, as well as *lys-4* in the monomicrobial infections was observed (Figure 2.8). It is known that lysozyme genes are differentially expressed due to exposure to both pathogenic and non-pathogenic bacteria in a species-specific pattern. During *C. albicans* infection, only *col-179* (a cuticular collagen gene) was significantly up-regulated (Figure 2.7). Interestingly, Sellegounder and co-workers (2018) found that this gene is important in the immune response against *P. aeruginosa*, however in our data sets it was only up-regulated in the *C. albicans* monomicrobial infection, indicating that it may be even more important in the defence against this pathogenic yeast. Noteworthy, these observed differences in gene expression might be due to the fact that these pathogens have evolved mechanisms to overcome host immune defences by inhibiting host defence signalling pathways and suppressing the expression of host defence effectors. Nevertheless, the exact immune function of most of these effectors still needs further clarification. Notably there are few limitations in this study that involves gene expression studies. The *C. elegans sek-1* strain used in our study lack a gene that encodes a conserved mitogen-activated protein kinase (MAPKK) involved in the innate immune response (Kim *et al*., 2002). According to literature, *sek-1* is required for responses to bacterial infection including *P. aeruginosa* and fungal infection including *Drechmeria coniospora* (Kim *et al*., 2002; Troemel *et al*., 2006; Pujol *et al*., 2008). Importantly, mutants that are known to be defective in the cell signaling or other defense molecules of *C. elegans* are revealed to be more susceptible to infection than the wild-type strains. Therefore the lack of *sek-1* does have an implication to our results in that disruption of genes involved in the p38-mediated innate immune signaling pathway such as *sek-1* can prevent the up-regulation of innate immunity genes.



Conditions	Genes	Fold change	*P*-value	Lower 95% CI	Upper 95% CI
***Candida albicans* vs control**	*spp-14*	-1.69	0.0047	0.45	-1.27
	ilys-5	-1.84	0.0400	0.30	-1.02
	spp-2	-2.51	0.0116	0.21	-1.29
	lys-4	-3.32	0.0151	0.13	-1.41
***Pseudomonas aeruginosa* vs control**	*elo-1*	-4.63	0.0307	0.07	-1.42
	spp-8	-5.56	0.0202	0.06	-1.91
	ilys-5	-6.78	0.0026	0.10	-4.44
	elo-5	-7.01	0.0223	0.04	-1.97
	spp-1	-7.99	0.0143	0.04	-2.71
	spp-3	-9.78	0.0014	0.07	-6.74
	fat-3	-9.87	0.0020	0.07	-6.36
	lys-4	-23.88	0.0035	0.02	-10.67
	spp-14	-29.08	0.0004	0.03	-21.60
Polymicrobial vs control	*elo-1*	-4.82	0.0293	0.06	-1.48
	spp-8	-5.78	0.0194	0.06	-1.99
	ilys-5	-7.05	0.0025	0.09	-4.62
	elo-5	-7.29	0.0214	0.04	-2.04

	spp-1	-8.31	0.0138	0.04	-2.82
	fat-1	-9.36	0.0085	0.04	-3.84
	fat-2	-10.02	0.0007	0.08	-7.71
	spp-3	-10.17	0.0014	0.07	-7.01
	fat-3	-10.26	0.0019	0.06	-6.61
	spp-2	-15.06	0,0027	0.04	-8.24
	lys-4	-24.84	0.0034	0.02	-11.09
	rps-4	-29.17	0.0001	0.03	-25.54
	spp-14	-30.24	0.0004	0.02	-22.47

Figure 2.8. Venn diagram depicting the overlap of down-regulated genes of *Caenorhabditis elegans* in response to *Candida albicans* and *Pseudomonas aeruginosa* infection. The table represents genes with a fold change of ≥ -1.5. *P*-values ≤ 0.05 indicate a significant difference from control, with lower and upper percentage confidence intervals (CI).

---

5. Conclusions

In summary, *C. elegans* infection model is capable to offer high-resolution longitudinal analysis as a powerful tool in order to unravel the contributions of the host and co-infecting bacteria and fungi. From the ample evidence supporting *C. albicans* and *P. aeruginosa* interactions, not only with their host, but also with each other, it is evident that the interaction is complex. Importantly, various virulence factors, including the yeast-hyphal morphogenesis, are known to affect and cause damage to the host, thus facilitating rapid and aggressive colonisation and infection. For instance, in this study, during *C. albicans* monomicrobial infection, we observed that the key component of C. *albicans* pathogenesis in mammals, hyphal formation, is also involved in nematode killing. Surprisingly, in the *C. elegans* model of co-infection, *P. aeruginosa* induced changes that lead to inhibition of hyphal formation by *C. albicans* and enhanced death of individual host animals. Interestingly, *C. elegans* infected with both *C. albicans and P. aeruginosa* showed the highest level of egg retention compared to nematodes infected by either *C. albicans or P. aeruginosa* alone. Presumably this egg retention is a mechanism used by the nematodes to protect their progeny due to co-infection being more virulent as compared to single infection. It is imperative to emphasise again the fact that the observed bacterial-fungal interactions *in vitro* can greatly differ from the observations made *in vivo*, either in animal model systems or in the human host. Therefore in this regard, new *in*

vivo tools and models including *C. elegans* used in this present study, will lead to more discoveries in order to complete the picture of these tri-kingdom interactions in disease.

In addition, FAs are widespread in most organisms and several findings suggest they may be utilised for defence purposes against *C. albicans* (Noverr *et al*., 2001; Murzyn *et al*., 2010; Muthamil *et al*., 2018; Prasath *et al*., 2019; Lee *et al*., 2021). However, despite the fact that the role of FAs in the interaction between single pathogens and hosts has been studied, a gap in knowledge still exists regarding the role of FAs in polymicrobial infection of *C. albicans* and *P. aeruginosa*. In our current study, we observed that the nematodes co-infected with both pathogens displayed changes in their FA composition. Interestingly, most of these observed changes reflect those caused by *P. aeruginosa* rather than *C. albicans*. In addition, we discovered a remarkably significant decrease in the relative percentage of 20:5n-3 and in our next chapters we will investigate the influence of this decrease in 20:5n-3 during infection. Lastly, in our gene expression studies, the differentially regulated genes differed between the different experimental conditions. We observed that changes in FA content corresponded with our studies of gene expression, revealing infection-induced changes in gene expression. Notably to our knowledge, this study was the first to investigate the influence of co-infection of *C. albicans* and *P. aeruginosa* on gene expression in *C. elegans*. Therefore, this opens a new avenue of research warranting further investigation. In addition, further analysis of the exact regulation of antimicrobial effector genes would be of great value for understanding their role in defence.

6. References

Aballay, A. & Ausubel, F. M. (2002). *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. *Curr Opin Microbiol* **5**, 97–101.

Alper, S., McBride, S. J., Lackford, B., Freedman, J. H. & Schwartz, D. A. (2007). Specificity and complexity of the *Caenorhabditis elegans* innate immune response. *Mol Cell Biol* **27** (15), 5544–53.

Balasubramanian, D., Schneper, L., Kumari, H. & Mathee, K. (2013). A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. *Nucleic Acids Res* **41**, 1–20.

Balla, K. M. & Troemel, E. R. (2013). *Caenorhabditis elegans* as a model for intracellular pathogen infection. *Cell Microbiol* **15**, 1313-1322.

Bergeron, A. C., Seman, B. G., Hammond, J. H., Archambault, L. S., Hogan, D. A. & Wheeler, R. T. (2017). *Candida* and *Pseudomonas* interact to enhance virulence of mucosal infection in transparent zebrafish. *Infect Immun* **85**.

Bomberger, J. M., MacEachran, D. P., Coutermarsh, B. A., Ye, S., O'Toole, G. A. & Stanton, B. A. (2009). Long-distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vesicles. *PLoS Pathog* **5**, e1000382.

Braun, B. R. & Johnson, A. D. (1997). Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science* **277**, 105–109.

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M. & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog* **3**, e18.

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

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T. & Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J Bacteriol* **183**, 5385–5394.

Chase, D. L. & Koelle, M. R. (2004). Genetic analysis of RGS protein function in *Caenorhabditis elegans*. *Methods Enzymol* **389**, 305–320.

Chotirmall, S. H., O'Donoghue, E., Bennett, K., Gunaratnam, C., O'Neill, S. J. & McElvaney, N. G. (2010). Sputum *Candida albicans* presages, F.E.V (1) decline and hospital-treated exacerbations in cystic fibrosis. *Chest* **138**, 1186–1195.

Corsi, A. K., Wightman, B. & Chalfie, M. (2015). A transparent window into biology: A primer on *Caenorhabditis elegans*. *Genetics* **200**, 387–407.

Couillault, C. & Ewbank, J. J. (2002). Diverse bacteria are pathogens of *Caenorhabditis* elegans. *Infect Immun* **70**, 4705-4707.

Couillault, C., Pujol, N., Reboul, J., Sabatier, L., Guichou, J. F., Kohara, Y. & Ewbank, J. J. (2004). TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat Immunol* **5**, 488– 494.

Darby, C., Cosma, C. L., Thomas, J. H. & Manoil, C. (1999). Lethal paralysis of *Caenorhabditis elegans* by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **96**, 15202–15207.

Dierking, K., Yang, W. & Schulenburg, H. (2016). Antimicrobial effectors in the nematode *Caenorhabditis elegans*: An outgroup to the Arthropoda. *Philos Trans R Soc Lond B Biol Sci* **371** (1695), 20150299.

Diaz, M. T., Alvarez, I., De La Fuente, J., Sanudo, C., Campo, M. M., Oliver, M. A., Fonti Furnols, M., Montossi, F., San Julian, R., Nute, G. R. & Caneque, V. (2005). Fatty acid composition of meat from typical lamb production systems of Spain, United Kingdom, Germany and Uruguay. *Meat Science* **71**, 256–263.

Driscoll, J. A., Brody, S. L. & Kollef, M. H. (2007). The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. *Drugs* **67**, 351–368.

Erb-Downward, J. R. & Huffnagle, G. B. (2007). *Cryptococcus neoformans* produces authentic prostaglandin E_2 without a cyclooxygenase. *Eukaryot Cell* **6**, 346–350.

Evans, E. A., Kawli, T. & Tan, M. W. (2008). *Pseudomonas aeruginosa* suppresses host immunity by activating the DAF-2 insulin-like signaling pathway in *Caenorhabditis elegans*. *PLoS Pathog* **4** (10), e1000175.

Fajardo, C., Costa, G., Nande, M., Martin, C., Martin, M. & Sanchez-Fortun, S. (2019). Heavy metals immobilization capability of two iron-based nanoparticles (nZVI and Fe3O4): soil and freshwater bioassays to assess ecotoxicological impact. *Sci Total Environ* **656**, 421-432.

Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**, 497-509.

Fourie, R., Ells, R., Swart, C. W., Sebolai, O. M., Albertyn, J. & Pohl, C. H. (2016). *Candida albicans* and *Pseudomonas aeruginosa* interaction, with focus on the role of eicosanoids. *Front Physiol* **7**, 64.

Fourie, R. & Pohl, C. H. (2019). Beyond antagonism: The interaction between *Candida* species and *Pseudomonas aeruginosa*. *J Fungi* (Basel) **5**, 34.

Gallagher, L. A. & Manoil, C. (2001). *Pseudomonas aeruginosa* PAO1 kills *Caenorhabditis elegans* by cyanide poisoning. *J Bacteriol* **183**, 6207–6214.

Gardner, M., Rosell, M. & Myers, E. M. (2013). Measuring the effects of bacteria on *C. elegans* behaviour using an egg retention assay. *J Vis Exp* **80**, e51203.

Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V., Murray, B. E., Calderwood, S. B. & Ausubel, F. M. (2001). A simple model host for identifying Gram-positive virulence factors. *Proc Natl Acad Sci USA* **98**, 10892-10897.

Geiss, G. K., Bumgarner, R. E., Birditt, B., Dahl, T., Dowidar, N. *et al*. (2008). Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* **26**, 317–325.

Cheung, G., Bae, J. S. & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence* **12** (1), 547–569.

Gravato-Nobre, M. J., Vaz, F., Filipe, S., Chalmers, R. & Hodgkin, J. (2016). The invertebrate lysozyme effector ILYS-3 is systemically activated in response to danger signals and confers antimicrobial protection in *C. elegans*. *PLoS Pathog* **12**, e1005826.

Han, S. K., Lee, D., Lee, H., Kim, D., Son, H. G. *et al*. (2016). OASIS 2: online application for survival analysis 2 with features for the analysis of maximal lifespan and health span in aging research. *Oncotarget* **7**, 56147–56152.

Henry, P., Owopetu, O., Adisa, D., Nguyen, T., Anthony, K., Ijoni-Animadu, D. *et al*. (2016). Fatty acids composition of *Caenorhabditis elegans* using accurate mass GCMS-QTOF. *J Environ Sci Health B* **51**, 546–552.

Hernandez-Chavez, M. J., Perez-Garcia, L. A., Nino-Vega, G. A. & Mora-Montes, H. M. (2017). Fungal strategies to evade the host immune recognition. *J Fungi (Basel)* **3**, 51.

Huffman, D. L., Abrami, L., Sasik, R., Corbeil, J., Van Der Goot, F. G. & Aroian, R. V. (2004). Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. *Proc Natl Acad Sci USA* **101**, 10995–11000.

Hur, S. J., Ye, B. W., Lee, J. L., Ha, Y. L., Park, G. B. & Joo, S. T. (2004). Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. *Meat Science* **66**, 771-775.

Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis Rep* **48**, 692.

Kadosh, D. & Mundodi, V. A. (2020). Re-evaluation of the relationship between morphology and pathogenicity in *Candida* species. *J Fungi (Basel)* **6** (1), 13.

Kim, D. H., Feinbaum, R., Alloing, G., Emerson, F. E., Garsin, D. A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M. W. & Ausubel, F. M. (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623-626.

Kim, S. H., Clark, S. T., Surendra, A., Copeland, J. K., Wang, P. W., Ammar, R., Collins, C., Tullis, D. E., Nislow, C., Hwang, D. M., Guttman, D. S. & Cowen, L. E. (2015). Global analysis of the fungal microbiome in cystic fibrosis patients reveals loss of function of the

transcriptional repressor *Nrg1* as a mechanism of pathogen adaptation. *PLoS Pathog* **11**, e1005308.

Kirienko, N. V., Kirienko, D. R., Larkins-Ford, J., Wählby, C., Ruvkun, G. & Ausubel, F. M. (2013). *Pseudomonas aeruginosa* disrupts *Caenorhabditis elegans* iron homeostasis, causing a hypoxic response and death. *Cell Host Microbe* **13**, 406–416.

Kong, C., Eng, S. A., Lim, M. P. & Nathan, S. (2016). Beyond traditional antimicrobials: A *Caenorhabditis elegans* model for discovery of novel anti-infectives. *Front Microbiol* **7**, 1956.

Krüger, W., Vielreicher, S., Kapitan, M., Jacobsen, I. D. & Niemiec, M. J. (2019). Fungal-bacterial interactions in health and disease. *Pathogens* **8** (2), 70.

Leclair, L. W. & Hogan, D. A. (2010). Mixed bacterial-fungal infections in the C.F. respiratory tract. *Med Mycol* **48**, 521522.

Lee, J. H., Kim, Y. G., Khadke, S. K. & Lee, J. (2021). Antibiofilm and antifungal activities of medium-chain fatty acids against *Candida albicans* via mimicking of the quorum-sensing molecule farnesol. *Microb Biotechnol* **14** (4), 1353-1366.

Lindsay, A. K. & Hogan, D. A. (2014). *Candida albicans*: molecular interactions with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Fungal Biol Rev* **28**, 85-96.

Lo, H. J., Kohler, J. R., Di Domenico, B., Loebenberg, D., Cacciapuoti, A. *et al*. (1997). Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90**, 939–949.

Lopez-Medina, E., Fan, D., Coughlin, L. A., Ho, E. X., Lamont, I. L., Reimmann, C., Hooper, L. V. & Koh, A, Y. (2015). *Candida albicans* inhibits *Pseudomonas aeruginosa* virulence through suppression of pyochelin and pyoverdine biosynthesis. *PLoS Pathog* **11**, e1005129.

Mahajan-Miklos, S., Tan, M. -W., Rahme, L. G. & Ausubel, F. M. (1999). Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa-Caenorhabditis elegans* pathogenesis model. *Cell* **96**, 47–56.

Mao, K., Ji, F., Breen, P., Sewell, A., Han, M., Sadreyev, R. & Ruvkun, G. (2019). Mitochondrial dysfunction in *C. elegans* activates mitochondrial relocalization and nuclear hormone receptor-dependent detoxification genes. *Cell Metab* **29** (5), 1182-1191.e4.

Mayer, F. L., Wilson, D. & Hube, B. (2013). *Candida albicans* pathogenicity mechanisms. *Virulence* **4**,119–128.

Miyata, S., Begun, J., Troemel, E. R. & Ausubel, F. M. (2008). DAF-16-dependent suppression of immunity during reproduction in *Caenorhabditis elegans*. *Genetics* **178**, 903–918.

Méar, J. B., Kipnis, E., Faure, E., Dessein, R., Schurtz, G., Faure, K. & Guery, B. (2013). *Candida albicans* and *Pseudomonas aeruginosa* interactions: more than an opportunistic criminal association? *Méd Mal Infect* **43**,146-151.

Mochii, M., Yoshida, S., Morita, K., Kohara, Y. & Ueno, N. (1999). Identification of transforming growth factor-beta-regulated genes in *Caenorhabditis elegans* by differential hybridization of arrayed cDNAs. *Proc Natl Acad Sci USA* **96** (26), 15020–25.

Mokoena, N. Z., Sebolai, O. M., Albertyn, J. & Pohl, C. H. (2020). Synthesis and function of fatty acids and oxylipins, with a focus on *Caenorhabditis elegans. Prostag Oth Lipid M* **148**, 106426.

Moy, T. I., Ball, A. R., Anklesaria, Z., Casadei, G., Lewis, K. & Ausubel, F. M. (2006). Identification of novel antimicrobials using a live-animal infection model. *Proc Natl Acad Sci USA* **103**, 10414-10419.

Moyes, D. L. & Naglik, J. R. (2011). Mucosal immunity and *Candida albicans* infection. *Clin Dev Immunol* **2011**, 346307.

Mukaremera, L., Lee, K. K., Mora-Montes, H. M. & Gow, N. A. R. (2017). *Candida albicans* yeast, pseudohyphal, and hyphal morphogenesis differentially affects immune recognition. *Front Immunol* **8**, 629.

Mullaney, B. C. & Ashrafi, K. (2009). *C. elegans* fat storage and metabolic regulation. *Biochim Biophys Acta* **1791**, 474-478.

Murzyn, A., Krasowska, A., Stefanowicz, P., Dziadkowiec, D. & Łukaszewicz, M. (2010). Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. *PLoS One* **5**, e12050.

Muthamil, S., Balasubramaniam, B., Balamurugan, K. & Pandian, S. K. (2018). Synergistic effect of quinic acid derived from syzygium cumini and undecanoic acid against *Candida* spp. biofilm and virulence. *Front Microbiol* **9**, 2835.

Nailis, H., Coenye, T., Van Nieuwerburgh, F., Deforce, D. & Nelis, H. J. (2006). Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. *BMC Mol Biol* **7**, 25.

Naglik, J. R., Challacombe, S. J. & Hube, B. (2003). *Candida albicans* secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* **67**, 400-428.

Nandakumar, M. & Tan, M. W (2008). Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity. *PLoS Genet* **4** (11), e1000273.

Nash, E. E., Peters, B. M., Fidel, P. L. & Noverr, M. C. (2015). Morphology-independent virulence of *Candida* species during polymicrobial intra-abdominal infections with *Staphylococcus aureus*. *Infect Immun* **84**, 90–98.

Nash, E. E., Peters, B. M., Palmer, G. E., Fidel, P. L. & Noverr, M. C. (2014). Morphogenesis is not required for *Candida albicans-Staphylococcus aureus* intra-abdominal infection-mediated dissemination and lethal sepsis. *Infect Immun* **82**, 3426–3435.

Neely, A. N., Law, E. J. & Holder, I. A. (1986). Increased susceptibility to lethal *Candida* infections in burned mice preinfected with *Pseudomonas aeruginosa* or pretreated with proteolytic enzymes. *Infect Immun* **52**, 200–204.

Nogueira, F., Sharghi, S., Kuchler, K. & Lion, T. (2019). Pathogenetic impact of bacterial-fungal interactions. *Microorganisms* **7** (10), 459.

Noverr, M. C., Phare, S. M., Toews, G. B., Coffey, M. J. & Huffnagle, G. B. (2001). Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect Immun* **69**, 2957–2963.

Peleg, A. Y., Hogan, D. A. & Mylonakis, E. (2010). Medically important bacterial-fungal interactions. *Nat Rev Microbiol* **8**, 340-349.

Peleg, A. Y., Tampakakis, E., Fuchs, B. B., Eliopoulos, G. M., Moellering, R. C. *et al*. (2008). Prokaryote-eukaryote interactions identified by using *Caenorhabditis elegans. Proc Natl Acad Sci USA* **105**, 14585-14590.

Pendleton, K. M., Huffnagle, G. B. & Dickson, R. P. (2017). The significance of *Candida* in the human respiratory tract: our evolving understanding. *Pathog Dis* **75**, ftx029.

Pierce, G. E. (2005). *Pseudomonas aeruginosa*, *Candida albicans*, and device-related nosocomial infections: implications, trends, and potential approaches for control. *J Ind Microbiol Biotechnol* **32**, 309-318.

Porta-de-la-Riva, M., Fontrodona, L., Villanueva, A. & Cerón, J. (2012). Basic *Caenorhabditis elegans* methods: synchronization and observation. *J Vis Exp* **64**, e4019.

Prasath, K. G., Sethupathy, S. & Pandian, S. K. (2019). Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. *J Proteomics* **208**, 103503.

Pujol, N., Cypowyj, S., Ziegler, K., Millet, A., Astrain, A. *et al*. (2008). Distinct innate immune responses to infection and wounding in the *C. elegans* epidermis. *Curr Biol* **18**, 481–489.

Pukkila-Worley, R., Peleg, A. Y., Tampakakis, E. & Mylonakis, E. (2009). *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryot Cell* **11**, 1750-1758.

Rhome, R. & Del Poeta, M. (2010). Sphingolipid signaling in fungal pathogens. *Adv Exp Med Biol* **688**, 232–237.

Roux, D., Gaudry, S., Dreyfuss, D., El-Benna, J., de Prost, N., Denamur, E., Saumon, G. & Ricard, J. (2009). *Candida albicans* impairs macrophage function and facilitates *Pseudomonas aeruginosa* pneumonia in rat. *Crit Care Med* **37**, 1062–1067.

Saville, S. P., Lazzell, A. L., Monteagudo, C. & Lopez-Ribot, J. L. (2003). Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell* **2**, 1053–1060.

Schafer, W. R. (2005). Egg-laying, *WormBook*, ed. The *C. elegans* Research Community, WormBook.

Schlecht, L. M., Peters, B. M., Krom, B. P., Freiberg, J. A., Hänsch, G. M., Filler, S. G., Jabra-Rizk, M. A. & Shirtliff, M. E. (2015). Systemic *Staphylococcus aureus* infection mediated by *Candida albicans* hyphal invasion of mucosal tissue. *Microbiology* **161**,168–181.

Schulenburg, H. & Boehnisch, C. (2008). Diversification and adaptive sequence evolution of *Caenorhabditis lysozymes* (Nematoda: Rhabditidae). *BMC Evol Biol* **8**, 114.

Sellegounder, D., Liu, Y., Wibisono, P., Chen, C. H., Leap, D. & Sun, J. (2019). Neuronal GPCR NPR-8 regulates *C. elegans* defense against pathogen infection. *Sci Adv* **5** (11), eaaw4717.

Shea, J. M., Kechichian, T. B., Luberto, C. & Del Poeta, M. (2006). The cryptococcal enzyme inositol phosphosphingolipid-phospholipase C confers resistance to the antifungal effects of macrophages and promotes fungal dissemination to the central nervous system. *Infect Immun* **74**, 5977–5988.

Slover, H. T. & Lanza, E. (1979). Quantitative analysis of food fatty acid by capillary gas chromatography. *J Am Oil Chem Soc* **112**, 1032-1040.

Spychalla, J. P., Kinney, A. J. & Browse, J. (1997). Identification of an animal omega-3 fatty acid desaturase by heterologous expression in arabidopsis. *Proc Natl Acad Sci USA* **94**, 1142–1147.

Sudbery, P., Gow, N. & Berman, J. (2004). The distinct morphogenic states of *Candida albicans*. *Trends Microbiol* **12**, 317–324.

Tan, M. W., Mahajan-Miklos, S. & Ausubel, F. M. (1999). Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci U S A* **96**, 715–720.

Tan, X., Chen, R., Zhu, S., Wang, H., Yan, D., Zhang, X. & Mylonakis, E. (2016). *Candida albicans* airway colonization facilitates subsequent *Acinetobacter baumannii* pneumonia in a rat model. *Antimicrob Agents Chemother* **60** (6), 3348–3354.

Tao, J., Hao, Y., Li, X., Yin, H., Nie, X., Zhang, J., Xu, B., Chen, Q. & Li, B. (2020). Systematic identification of housekeeping genes possibly used as references in *Caenorhabditis elegans* by large-scale data integration. *Cells* **9** (3), 786.

Thibane, V. S., Ells, R., Hugo, A., Albertyn, J., van Rensburg, W. J., Van Wyk, P. W., Kock, J. L. & Pohl, C. H. (2012). Polyunsaturated fatty acids cause apoptosis in *C. albicans* and *C. dubliniensis* biofilms. *Biochim Biophys Acta* **1820**, 1463-1468.

Thomas, J. H. (2007). Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. *PLoS Genet* **3**, e67.

Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M. & Kim, D. H. (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* **2** (11), e183.

Valenza, G., Tappe, D., Turnwald, D., Frosch, M., Konig, C., Hebestreit, H. & Abele-Horn, M. (2008). Prevalence and antimicrobial susceptibility of microorganisms isolated from sputa of patients with cystic fibrosis. *J Cystic Fibrosis* **7**, 123-127.

Watts, J. L. (2009). Fat synthesis and adiposity regulation in *Caenorhabditis elegans*. *Trends Endocrinol Metab* **20**, 58–65.

Watts, J. L. & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **99**, 5854–5859.

Watts, J. L. & Ristow. M. (2017). Lipid and carbohydrate metabolism in *Caenorhabditis elegans*. *Genetics* **207** (2), 413–46.

Wang, M., Chen, H., Gu, Z., Zhang, H., Chen, W. & Chen, Y. Q. (2013). ω-3 fatty acid desaturases from microorganisms: structure, function, evolution, and biotechnological use. *Appl Microbiol Biotechnol* **97** (24), 10255–62.

Xu, H., Sobue, T., Thompson, A., Xie, Z., Poon, K., Ricker, A., Cervantes, J., Diaz, P. I. & Dongari-Bagtzoglou, A. (2014a). Streptococcal co-infection augments *Candida* pathogenicity by amplifying the mucosal inflammatory response. *Cell Microbiol* **16**, 214-231.

Xu, L., Wang, F., Shen, Y., Hou, H., Liu, W., Liu, C. *et al*. (2014b). *Pseudomonas aeruginosa* inhibits the growth of pathogenic fungi: *in vitro* and *in vivo* studies. *Exp Ther Med* **7**, 1516-1520.

Yadav, J., El Hassani, M., Sodhi, J., Lauschke, V. M., Hartman, J. H. & Russell, L. E. (2021). Recent developments in *in vitro* and *in vivo* models for improved translation of preclinical pharmacokinetics and pharmacodynamics data. *Drug Metab Rev* **53** (2), 207-233.

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X. & Liang, B. (2013). Comparative genomics and functional study of lipid metabolic genes in *Caenorhabditis elegans. BMC Genomics* **14**, 164.

Zhou, X. R., Green, A. G. & Singh, S. P. (2011). *Caenorhabditis elegans* Δ12- desaturase FAT-2 is a bifunctional desaturase able to desaturate a diverse range of fatty acid substrates at the Δ12 and Δ15 positions. *J Biol Chem* **286** (51), 43644–50.

Chapter 3

The influence of long-chain polyunsaturated fatty acids on behaviour, susceptibility to infection and egg retention in *Caenorhabditis elegans*

1. Abstract

Due to a limited set of antimicrobials available and problems in early diagnosis, bacteria-fungal polymicrobial infections caused by *Candida albicans* and *Pseudomonas aeruginosa* are among the most common hospital-acquired infections with high mortality rates. Interestingly, fatty acids (FAs) are known to possess antimicrobial effect on numerous microbial pathogens. Using the *Caenorhabditis elegans-C. albicans-P. aeruginosa* host-pathogen system, we studied the role for polyunsaturated fatty acids (PUFAs), arachidonic acid (AA) and eicosapentaenoic acid (EPA) on susceptibility to infections. Firstly, we monitored the chemotaxis behaviour of *C. elegans* towards AA and EPA sodium salts, in order to compensate for any bias in *C. elegans* behaviour towards these FAs. Thereafter, we determined the influence of mono- and polymicrobial infected AA and EPA supplemented nematodes on FA composition and the changes in the PUFA biosynthesis pathway using lipid extraction and gas chromatography. We then determined the influence of FAs on the survival of nematodes with mono- and polymicrobial infection with *C. albicans* and *P. aeruginosa*, as well as egg retention of infected nematodes using egg-in-worm assay. In this study, we demonstrated that *C. elegans* does not avoid the FAs to any statistically significant degree, thus it is expected that any changes in susceptibility towards these FAs would not be as a result of avoidance of *E. coli* supplemented with the PUFAs. Supplementation with either AA or EPA resulted in changes in FA profiles and significantly influenced survival of infected nematodes. EPA supplementation effectively reduced *C. albicans* virulence and inhibited hyphal formation, thus leading to a partial rescue of pathogen susceptibility. However, this was not the case for *P. aeruginosa* infections. Finally, we uncovered that the degree of egg retention elicited by *C. albicans* and *P. aeruginosa* was influenced by PUFA supplementation. Together, these data add to the known antimicrobial effects of PUFAs, and suggest a link between FAs and host susceptibility to microbial infection in this model.

Key word: Antimicrobials, polymicrobial infections, *Candida albicans, Pseudomonas aeruginosa, Caenorhabditis elegans,* arachidonic acid, eicosapentaenoic acid, supplementation, chemotaxis

2. Introduction

One of the central challenges found in biology is understanding the cellular functions of majority of the complex lipids that exist in animal cells. A diverse group of fatty acids (FAs) are known to possess a protective role in nature as antimicrobial agents and ecological modulators that control microbial biofilms and virulence (Kumar *et al*., 2020). Polyunsaturated fatty acids (PUFAs), including omega-3 (n-3) and omega-6 (n-6) FAs have pleiotropic effects with many metabolic benefits such as improving the immune system, the cardiovascular, and the neurologic health in mammals (Ruxton *et al*., 2004). The influence of lipids on the immune response in mammals is multifaceted, and majority of the studies point towards the beneficial effects of long chain fatty acids (LC-FAs) in immunity. However, the mode of action that PUFAs use to modulate innate immunity and also the effects of PUFA deficiencies on innate immune functions still needs more studies. Thus far, discoveries made through the use of different model organisms have made a significant impact on human medicine. For instance, research using *Caenorhabditis elegans* has played a crucial in investigating the genetic pathways underlying signal transduction pathways, longevity, and programmed cell death that transpire during development and carcinogenesis (Corsi *et al*., 2015). Interestingly, this research has thus resulted in the discovery of novel therapeutics and drug targets in humans. In addition to these benefits observed in disease control and prevention, to date, most of the natural PUFAs are becoming increasingly relevant as antimicrobial agents (Desbois and Smith, 2010). For instance, significant antimicrobial activities of purified linoleic acid (LA, 18:2n-6), arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3), have been reported against various pathogenic bacteria including *Neisseria gonorrhoeae* (Bergsson *et al*., 1999), *Staphylococcus aureus* (Shin *et al*., 2007), *Pseudomonas aeruginosa* (Tiesset *et al*., 2009), *Burkholderia cenocepacia* (Mil-Homens *et al*., 2010), and *Helicobacter pylori* (Correia *et al*., 2012).

Recent studies indicate that FAs also hold great potential as antibiofilm agents, as many FAs have been identified to selectively inhibit or disrupt the formation of biofilms by various microbial pathogens, including *Candida albicans* (Murzyn, *et al*., 2010; Muthamil, *et al*., 2018; Prasath *et al.,* 2019), *S. aureus* (Davies and Marques, 2009; Kim *et al*., 2018), *P. aeruginosa* (Inoue *et al*., 2008; Wenderska *et al*., 2011), *Serratia marcescens*, *B. cenocepacia*, and *Vibrio* spp. (Santhakumari *et al*., 2017; Ramanathan *et al*., 2018; Cui *et al*., 2019). Moreover, *in vivo* studies using a mouse model, demonstrated that an oral supplementation with n-3 FAs can also be effective at controlling microbial infections (Tiesset *et al*., 2009; Caron *et al*., 2015). Although these and other studies suggest important roles of LC-PUFAs in pathogenesis, the influence of these LC-PUFAs on *C. albicans* and *P. aeruginosa* polymicrobial infections remains unstudied. To address this lack of functional evidence, we used *C*. *elegans* as an

infection model and analysed the influence of FA supplementation on monomicrobial and polymicrobial infections of *C. albicans* and *P. aeruginosa*.

3. Materials and methods

3.1. Strains used

Caenorhabditis elegans glp-4; sek-1 hermaphrodites, obtained from the *Caenorhabditis* Genetic Centre, College of Biological Sciences, University of Minnesota, were propagated on Nematode Growth Medium (NGM) (2.5 g/L peptone, 3 g/L sodium chloride, 17 g/L agar) spotted with *Escherichia coli* OP50 as food source, using the technique previously described by Brenner (1974). In all experiments, we used *E. coli* OP50 as our control. *Candida albicans* SC5314 was maintained on yeast extract-peptone-dextrose (YPD) agar (5 g/L peptone, 3 g/L yeast extract, 10 g/L glucose, 16 g/L agar) at 30 °C, while *P. aeruginosa* PAO1 was maintained in Luria Bertani (LB) broth (10 g/L sodium chloride, 5 g/L yeast extract, 10 g/L tryptone) at 37 °C.

3.2. Chemotaxis behaviour of nematodes

Synchronisation of the nematodes for the chemotaxis assays was performed as previously described (Wood, 1987). The chemotaxis assay protocol was adapted from Margie *et al*. (2013). Briefly, the assay was performed in 5 cm petri dishes, containing 12 ml of NGM agar. Each petri dish was divided into 4 quadrants. During the assay, 8 µl of nematodes, washed with M9 buffer (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, 0.25 g/L $MgSO_4.7H_2O$) were transferred with a pipette to a 6 mm radius inner circle region of the plate. Stock solutions of 0.1 M eicosapentaenoic acid (EPA) and arachidonic acid (AA) sodium salts (Sigma-Aldrich) were prepared in ethanol and diluted in ethanol to 0.01 mM AA or EPA. Thereafter, 2 µl of the test compounds were spotted on two opposite quadrants of the petri dish and 2 µl diluted ethanol control was spotted on the two remaining quadrants. All spots also contained 2 µl of 0.5 M sodium azide (Sigma-Aldrich), which was used as an anaesthetic to retain the nematodes. Nematodes were allowed to roam for 1 h and 24 h before quantifying the results. Nematodes were excluded from the analysis if they failed to clear the inner circle, were sitting on the marked lines or less than 2 body lengths away from those lines. Nematodes that were located on the edges or walls of the plates were counted as part of the corresponding quadrant. For each experiment, a chemotaxis index (CI) was calculated using the following formula:

CI = (number of nematodes in both test quadrants - number of nematodes in both control quadrants)/(total number of scored nematodes).

A score of +1.0 corresponds to maximal attraction, whereas a score of -1.0 means total repulsion, and results close to 0 indicate a lack of response to the compound. Chemotaxis assays were performed in triplicate for each condition, and indexes for each replicate were calculated.

3.3. Fatty acid supplementation of nematodes

Nematode Growth Medium (NGM) agar was prepared with addition of 10 ml of 0.1% Tergitol (NP-40), which allows for even distribution of FAs through the entire plate and more efficient uptake of the FAs by *E. coli* and the nematodes (Deline *et al*., 2013). Agar was cooled to 45-50 °C and 0.01 mM of either AA or EPA sodium salt was added slowly. Plates were poured immediately, covered to dry in the dark for 24 h, thereafter seeded with *E. coli* OP50, and incubated for 24 h in the dark at room temperature. Synchronized L4 *C. elegans* nematodes that were grown for 4 days on NGM agar plates with or without 0.01 mM FAs, seeded with *E. coli* OP50, were carefully harvested and washed three times with sterile M9 buffer. Subsequently, the nematodes were transferred into sterile petri-dishes containing 10 ml liquid medium (80% M9 buffer, 20% Brain Heart Infusion [(BHI) (7.8 g/L brain extract, 9.7 g/L heart extract, 2.5 g/L disodium phosphate, 2.0 g/L dextrose)] and 90 µg/ml kanamycin), thereafter incubated at 25 °C for 24 h.

3.4. Influence of infection on fatty acid composition of supplemented nematodes

In order to study the effect of infection, the same procedure as described in chapter 2 section 3.3 and 3.4 was followed, with the only exception being that synchronised L4 nematodes were infected with either or both *C. albicans* and *P. aeruginosa* after growth on NGM with or without 0.01 mM AA or EPA, seeded with *E. coli* OP50. Infections were carried out according to the modified *C. elegans* liquid medium killing assay protocol by Breger *et al*. (2007). For monomicrobial infections, roughly more than 500 washed synchronized L4 *C. elegans* nematodes, grown on sixty NGM agar plates with or without 0.01 mM FA sodium salt seeded with *E. coli* OP50, were placed on the centre of either *C. albicans* or *P. aeruginosa* lawn grown on BHI agar as well as the control nematodes then were incubated at 25 °C for 4 h. For polymicrobial infections, BHI agar plates with *C. albicans* infected nematodes were first incubated for 2 h at 25 °C, then washed off and further transferred to *P. aeruginosa* lawn grown on BHI plates and incubated for 2 h at 25 °C. After incubation, nematodes were carefully

washed off the BHI agar plates with 20 ml of sterile M9 buffer and transferred to 50 ml conical tube. Nematodes were thoroughly washed with M9 buffer four times. Any microbial contaminants, which may confound the infection process, were removed via sucrose floatation during the washing step (Jenkins, 1964). Subsequently, the nematodes were transferred into sterile petri-dishes containing 10 ml liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) and were incubated at 25 °C for 24 h. Following incubation, nematodes were washed by centrifugation at 4000 *g* for 2 min, the supernatant gently aspirated, and the pellet containing the nematodes pulverised with a mortar and pestle to break open the nematodes for the release of their intracellular components. The total lipids of the nematodes were extracted overnight using chloroform/methanol (2:1 v/v) solvent system (Folch *et al*., 1957). Thereafter, the extract was filtered, and the solvent phase, containing the lipids was removed, dried under nitrogen and stored at -80 °C prior to analysis using gas chromatography (GC).

3.5. Gas chromatography analysis

Fatty acids were transesterified to form methyl esters (FAMEs) using 0.5 N NaOH and 14% boron trifluoride in methanol (Slover and Lanza, 1979; Hur *et al*., 2004; Diaz *et al*., 2005). Fatty acid methyl esters were quantified using a Varian 430 flame ionization gas chromatography (GC), with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses). All the analysis were performed using an initial isothermic period (40 °C for 2 min). Thereafter, the temperature was increased at a rate of 4 °C/min to 230 °C. This was followed by an isothermic period of 230 °C for 10 min. The FAMEs were then dissolved in *n*-hexane then 1 µl was injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were both maintained at a constant temperature of 250 °C. The hydrogen, at 45 psi, served as the carrier gas, while nitrogen served as the make-up gas. Finally, the chromatograms were recorded using the Galaxy Chromatography Software. The FAME samples were identified by comparing the retention times of authentic standards (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich). All other reagents and solvents that were used were of analytical grade and obtained from Merck Chemicals. The unsaturation indixes of the extracted lipids of the control and supplemented was calculated as follows: Unsaturation Index = 1×[% monoenoic fatty acids] + 2×[% dienoic fatty acids] + 3×[% trienoic fatty acids] + 4×[% tetraenoic fatty acids] + 5×[% pentaenoic fatty acids] (Thibane *et al*., 2012).

3.6. Influence of infection on the survival of supplemented nematodes

Roughly 400 to 500 washed synchronized L4 *C. elegans* nematodes, grown on sixty NGM agar plates with or without 0.01 mM AA or EPA seeded with *E. coli* OP50, were placed on the centre of either *C. albicans* or *P. aeruginosa* lawn grown on BHI agar. For monomicrobial infections, BHI agar plates with infected nematodes as well as control nematodes, feeding on *E. coli* OP50, were incubated at 25 °C for 4 h. While, for polymicrobial infections, BHI agar plates with *C. albicans* infected nematodes were first incubated for 2 h at 25 °C, then washed off and further transferred to *P. aeruginosa* lawn grown on BHI plates and incubated for 2 h at 25 °C. After incubation, nematodes were carefully washed off the BHI agar plates with 6 ml of sterile M9 buffer and transferred to 15 ml conical tube. Nematodes were thoroughly washed with M9 buffer four times. Any microbial contaminants, which may confound the infection process, was removed via sucrose floatation during the washing step (Jenkins, 1964). About 60 to 70 nematodes were transferred into 2 ml of liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) in a single well of a six-well tissue culture plate. Nematodes were daily monitored by scoring them as either alive, dead or dead with hyphal formation. If nematodes did not show any movement in response to mechanical stimulation, they were considered dead and thus removed from liquid medium assay (Breger *et al*., 2007).

3.7. Egg-in-worm assay

The egg-in-worm (EIW) assay of the infected nematodes were carried out according to Gardner *et al*. (2013). Freshly grown *C. albicans* was inoculated into 5 ml of YPD broth then incubated at 37 °C overnight. Similarly, *P. aeruginosa* was inoculated into 5 ml of LB broth and incubated at 37 °C overnight. Cell densities were adjusted to final cell concentration of approximately $l0^6$ cells/ml. Lawns of either or both *C. albicans* and *P. aeruginosa* were prepared separately by plating 100 µl of the yeast or bacterial strains as 10 mm square lawns on a large (100 mm x 15 mm) BHI agar plate, then incubated at 37 °C for 24 h. Synchronized L4 *C. elegans* nematodes, growing on two large NGM agar plates supplemented with either AA or EPA and without FAs, thereafter, seeded with *E. coli* OP50 were washed off carefully with sterile M9 buffer. About 15 - 20 L4 *C. elegans* with and without FAs were picked and placed onto the centre of a lawn of either or both bacteria and yeast. Plates were then incubated for 40 h at 15 °C. A 20% bleach solution was prepared mixing a commercial bleach (6.0% sodium hypochlorite) with the appropriate volume of distilled water. Thereafter, 10 µl drops of bleach solution were added to ten distinct locations on a plastic petri dish lid. Using a pipette, one nematode was transferred into each bleach drop. The cuticle of the nematode was allowed to dissolve for approximately 10 min or until the nematode burst open, expelling

the eggs. The number of retained eggs was easily quantified by counting while viewing under Olympus stereo dissecting microscope.

3.8. Statistical analyses

All experiments were executed in triplicate and repeated three times. The average and standard deviation was calculated. The student *t*-test was carried out to determine statistically significant differences between data sets. A *P*-value of ≤ 0.05 was considered significant. The *C*. *elegans* survival was assessed using the Kaplan-Meier method and differences were determined with the log-rank test using OASIS 2 with statistical analyses performed using two-way ANOVA with Bonferroni correction (Han *et al*., 2016).

4. Results and Discussions

4.1. The effect of long-chain polyunsaturated fatty acids on chemotaxis behaviour of *C. elegans*

Several forms of behavioural plasticity have been reported in *C. elegans* such as chemotaxis which is used to assay *C. elegans* attraction from or avoidance towards soluble compounds (Wicks *et al*., 2000). As depicted in figure 3.1, we discovered that after time intervals of 1 h and 24 h, the nematodes were slightly attracted to ethanol (solvent control), with a CI of approximately 0.5. The near zero CI values obtained for AA [CI = 0.06±0.08 (1 h); CI = -0.08±0.05 (24 h)] and EPA [CI = 0.02±0.06 (1 h); CI = 0.07±0.10 (24 h)] were significantly different ($P < 0.05$) from the control values. As stated in literature, a + 1.0 score corresponds to maximal attraction, whereas a -1.0 score means total repulsion, and results close to zero indicate a lack of response to the compound (Margie *et al*., 2013). This indicates that the nematodes do not avoid either AA or EPA in this scenario. These results are important as they prevent skewing of the infection data by supplementation with these FAs, ensuring that supplementing FAs will not be avoided, resulting in lack of feeding by the nematodes.

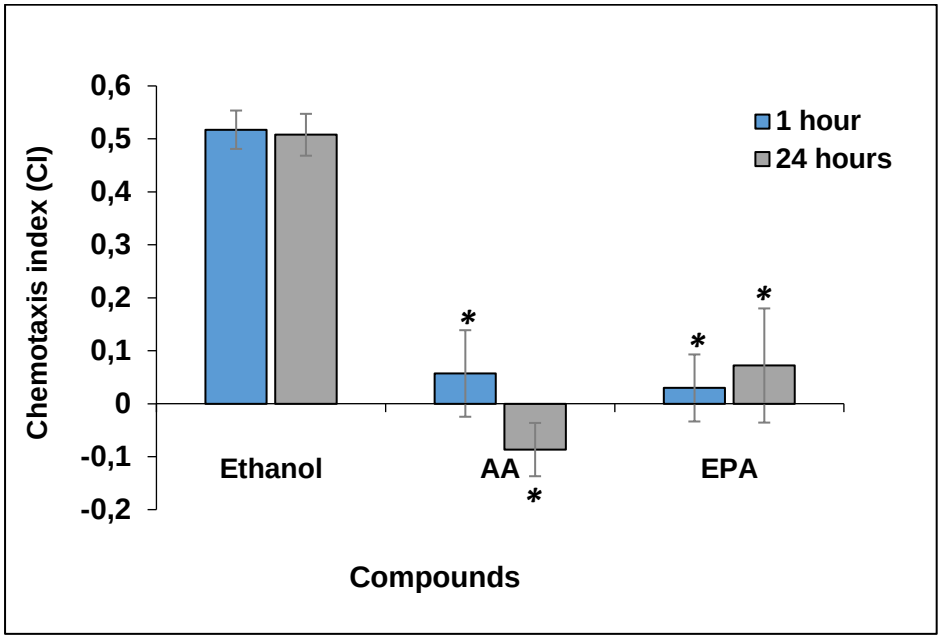


Figure 3.1. Chemotaxis indexes calculated for *Caenorhabditis elegans* exposed to arachidonic acid (AA) or eicosapentaenoic acid (EPA) for 1 hour and 24 hours. Ethanol was used as a control. Values represents the mean of three independent experiments and error bars represents the standard deviations. Asterisk (*) indicate $P < 0.05$ compared to control.

4.2. Influence of PUFA supplementation on nematode fatty acid profiles

Fatty acid supplementation is an effective means to alter the FA composition of nematodes and can also be used to rescue defects in FA-deficient mutants (Deline *et al*., 2013). According to literature, supplementation of most FAs has no detrimental effects on wild-type nematodes (Brock *et al*., 2006; Webster *et al*., 2013; Deline *et al*., 2013). However, specific n-6 FAs, especially dihomo-gamma linolenic acid (20:3n-6), can permanently cause destruction of the germ cells of *C. elegans* (Brock *et al*., 2006; Webster *et al*., 2013; Deline *et al*., 2013). Therefore, given the previously observed link between altered FA profiles and infections in *C. elegans*, we investigated the potential of FAs to act as antimicrobial agents. We first tested the effect of AA and EPA sodium salts supplementation on the FA profiles of nematodes growing on *E. coli* OP50. We discovered that the total lipids of control unsupplemented nematodes had a higher unsaturation index compared to AA and EPA supplemented nematodes, although this difference was not statistically significant (Figure 3.2A). Moreover, the change in FA compositions of the nematodes was depicted in figure 3.2B, demonstrating that supplementation with AA and EPA changes the relative amount of PUFAs in the total lipids of the nematodes. Surprisingly supplementation with either AA or EPA did not increase

the FA composition percentages of these PUFAs in the nematodes, but caused a significant decrease in the relative percentage of EPA, with a concomitant increase in the relative percentage 18:2n-6 ($P < 0.005$) compared to unsupplemented nematodes (Figure 3.2B). Similar results were observed by Bouyanfif (2019), in wild-type nematodes, where supplementation with EPA did not cause any significant change in EPA composition, although a decreasing trend was seen for EPA as well as a non-significant increase in α-linolenic acid (18:3n-3) similar to our results. Noteworthy, supplementation of the *C. elegans* diet is limited by the ability of the bacterial food source, such as *E. coli* OP50, to take up and incorporate the FAs into their lipids.

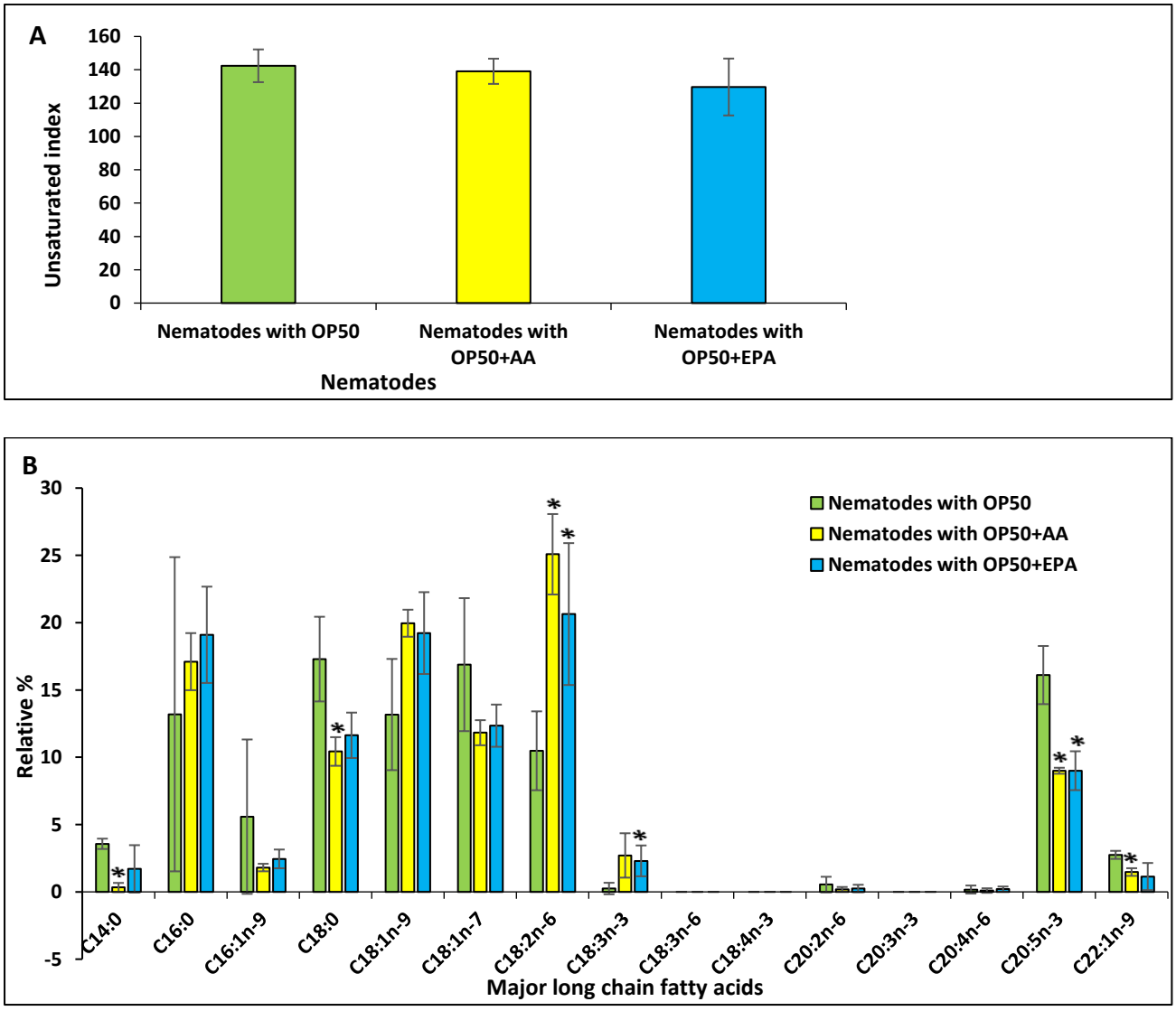


Figure 3.2. (A) Effect of arachidonic acid (AA) and eicosapentaenoic acid (EPA) supplementation on unsaturation index of *Caenorhabditis elegans* on *Escherichia coli* OP50. (B) Effect of AA and EPA supplementation on fatty acid composition of major long chain fatty

acids of *C. elegans*, as determined by gas chromatography analysis. Values represents the mean of three independent experiments and error bars represents the standard deviations. Asterisk (*) indicate $P < 0.05$ compared to unsupplemented nematodes.

4.3. Influence of infection on nematode fatty acid profiles

We next examined the influence of infection on FA profiles of unsupplemented nematodes and nematodes supplemented with either AA or EPA. As depicted in figure 3.3A, in terms of the unsupplemented nematodes (as indicated in chapter 2 as well), infection by either or both *C. albicans* and *P. aeruginosa* caused a significant decrease in the relative percentage of EPA. The highest decrease was seen during co-infection with both pathogens ($P = 0.0003$). Interestingly for most of the other FAs, the changes due to *C. albicans* or *P. aeruginosa* single infection were not statistically significant (Figure 3.3A). By contrast, co-infection with both pathogens caused changes in most of the other FAs, especially a significant increase in the relative percentage of vaccenic acid (18:1n-7) ($P = 0.0013$) and 18:3n-3 ($P = 0.030$) (Figure 3.3A).

Research showed that the manipulation of lipid metabolism by supplementation of different dietary FAs can extend *C. elegans* lifespan (Qi *et al*., 2017; Papsdorf and Brunet, 2018). One of the mechanisms which dietary FAs influence lifespan is through the activation of transcription factor network (Qi *et al*., 2017). For example, supplementation with 18:3n-3 extends lifespan of *C. elegans* through the NHR-49/PPARα and the SKN-1/NRF transcription factors regulation (Qi *et al*., 2017). The 18:3n-3 directly binds to NHR-49 thus activating it, while also it indirectly leads to the activation of SKN-1/NRF via its hydroxylated or peroxidised counterparts such as 9-hydroperoxyoctadeca-10, 12, 15-trienoic acid [9(S)-HpOTrE] (Qi *et al*., 2017). In addition, the role of monounsaturated fatty acids (MUFAs), such as 18:1n-7, was revealed through studies done by Han and colleagues (2017). They revealed that MUFAs, palmitoleic acid (16:1n-9) and 18:1n-7, were significantly elevated at all stages of *C. elegans* life cycle tested. The accumulation of these MUFAs was necessary for the lifespan extension of trimethylation of lysine 4 on histone H3 (H3K4me3) methyltransferase deficient nematodes (Han *et al*., 2017). Moreover, dietary supplementation of individual MUFAs [16:1n-9, 18:1n-7 or oleic acid (18:1n-9)] was sufficient to extend lifespan. Thus both 18:1n-7 and 18:3n-3 may be important in survival of *C. elegans.*

Furthermore, in our studies, we observed an overall similar trend in changes in FA profiles of infected nematodes, regardless of FA supplementation (Figure 3.3B and 3.3C). The data indicates that infection with *C. albicans* alone has minimal effect on the relative percentages of the AA- and EPA supplemented nematodes, compared to the unsupplemented nematodes.

However, similar to the unsupplemented nematodes, the most significant changes in FA profiles were observed for infections by either *P. aeruginosa* alone, or in combination with *C. albicans*. These changes entailed significant decreases in relative percentage of stearic acid (18:0), 18:1n-9, 18:2n-6 and 20:5n-3, and significant increases of palmitic acid (16:0), 16:1n-9 and again especially 18:1n-7 (Figure 3.3B and 3.3C). The significant decreases in relative percentage of 18:0 and 18:1n-9 were interesting. Previously, Nandakumar *et al*. (2008) showed that two PUFAs, γ-linolenic acid (18:3n-6) and 18:0, are required for the basal expression of innate immune effectors and pathogen resistance in *C*. *elegans.* Anderson and colleagues (2019) further showed that 18:1n-9 is necessary for innate immune activation and resistance to bacterial infection in a manner distinct from the effects of 18:0 and 18:3n-6. In addition, although it was discovered that 18:1n-9 is sufficient to extend the lifespan of nematodes that were grown under standard laboratory conditions, treatment with 18:1n-9 was not sufficient to provide protection during bacterial infection, but was required for proper immune gene transcription (Han *et al*., 2017; Anderson *et al*., 2019). Specifically, 18:1n-9 is important for the pathogen-mediated induction of immune effectors that are downstream of the p38 mitogen-activated protein kinase PMK-1 (p38 MAPK PMK-1) pathway and for genes that are regulated by the bZIP transcription factor ZIP-2, which functions independently of the canonical PMK-1 pathway to mediate an early transcriptional response to *P*. *aeruginosa* infection (Estes *et al*., 2010). Consistent with these data Anderson *et al*. (2019) supplementation studies indicated that 18:1n-9 treatment itself does not activate immune gene transcription. Thus, 18:1n-9 is unlikely to be a signal of immune activation in *C*. *elegans*, but rather functions as a licensing factor for the elaboration of anti-pathogen responses (Anderson *et al*., 2019). Disruption of 18:1n-9 biosynthesis alters membrane fluidity, which has pleiotropic consequences on membrane-bound organelles, including activating stress pathways associated with endoplasmic reticulum dysfunction (Hou *et al*., 2014). Indeed, alterations of membrane fluidity have been linked to activation of G protein-coupled receptors (Yang *et al*., 2005; Bodhicharla *et al*., 2018). Thus, changing the 18:1n-9 content in *C*. *elegans* may modulate the ability of the host to mount protective defence responses, either directly or by disrupting lipid-protein interactions that are essential for immune pathway activation. These findings present a previously unappreciated link between a highly abundant FA and immune activation, which may represent an ancient connection between body energy stores and susceptibility to bacterial infection.

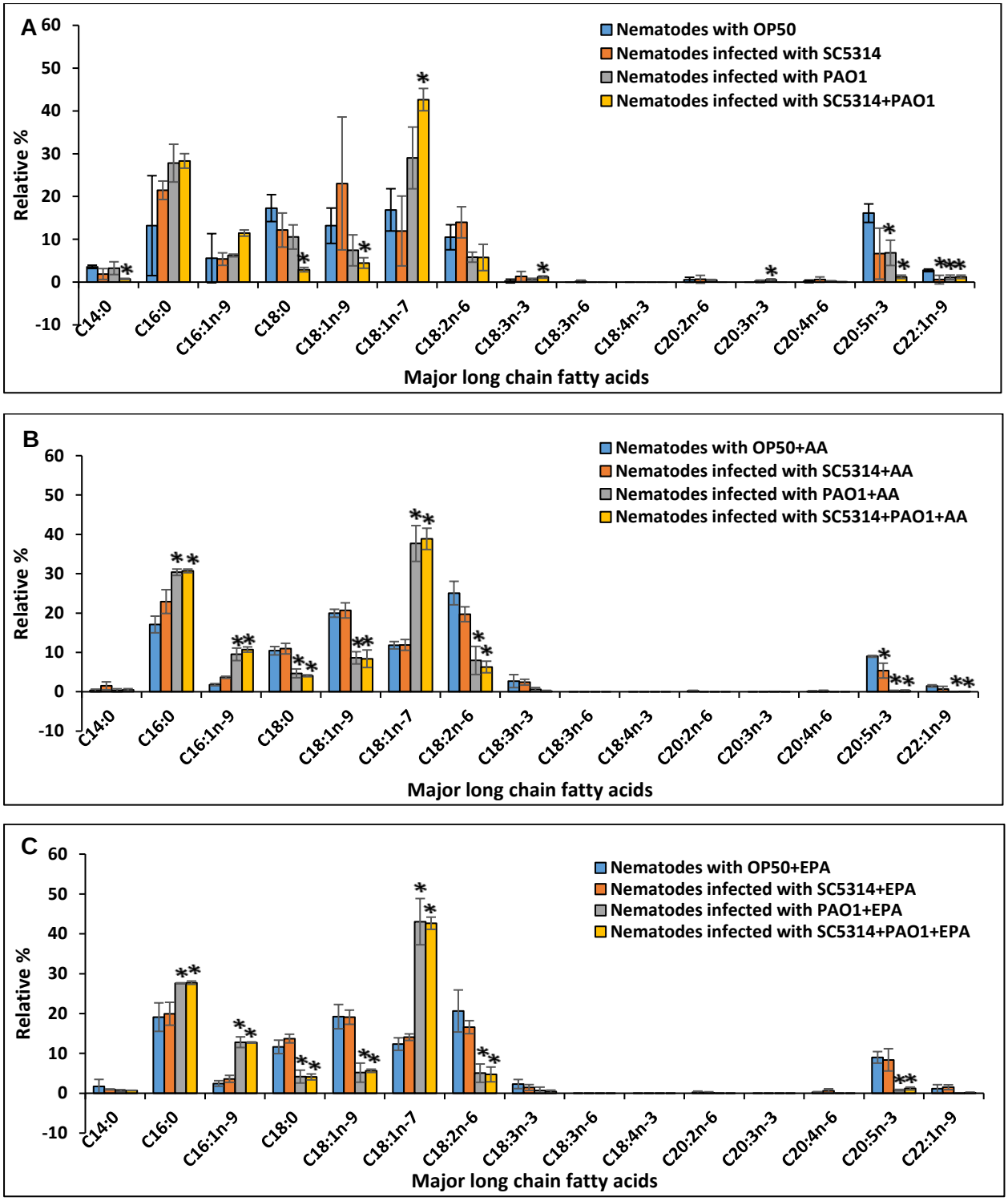


Figure 3.3. Effect of infection on fatty acid profiles of (A) unsupplemented nematodes, (B) nematodes supplemented with arachidonic acid (AA) and (C) nematodes supplemented with eicosapentaenoic acid (EPA). Values represents the mean of three independent experiments and error bars represents the standard deviations. Asterisk (*) indicate $P < 0.05$ compared to uninfected nematodes.

4.4. The influence of supplemented long-chain polyunsaturated fatty acids on susceptibility to infection

4.4.1. Eicosapentaenoic acid supplementation influences survival of *C. albicans*-infected nematodes by inhibiting hyphal formation

Fatty acid metabolism is known to play an important role in many physiological and pathological processes (Anderson *et al*., 2019). Furthermore, previous studies showed that a large number of lipid molecules, such as FAs and eicosanoids can modulate the yeast to hyphal transition in *C. albicans* (McLain *et al*., 2000; Noverr and Huffnagle, 2004; Clement *et al*., 2007; Murzyn *et al*., 2010). For instance, FAs, including butyric, capric, lauric, palmitoleic, oleic, linoleic, conjugated linoleic, and AA inhibited the yeast to hyphal transition induced under various conditions (McLain *et al*., 2000; Noverr and Huffnagle, 2004; Clement *et al*., 2007; Murzyn *et al*., 2010). To elucidate the role of PUFAs in *C. elegans* response to *C. albicans*, nematodes were raised in the presence of dietary supplied AA and EPA and infected with the yeast. Figure 3.4 and Table 3.1 show the results and statistical analysis of the completed lifespan assays. We observed that the survival of AA supplemented nematodes, infected with *C. albicans* SC5314, was not significantly different from unsupplemented, infected nematodes (P = 1) (Figure 3.4A). *C. albicans* SC5314 infection caused death in 50% of the nematodes after 2 days and 100% mortality after 7 days. However, EPA supplementation, prior to infection with *C. albicans* SC5314, significantly extended the time needed to kill 50% of the nematodes to 4 days, although it did not influence the time needed to kill 100% of the nematodes (Figure 3.4A). This indicates that although the changes in FA profiles caused by EPA supplementation are similar to those caused by AA supplementation, EPA supplementation, in contrast to AA supplementation, influences the early stages of infection in this model. This is important since Pukkila *et al*. (2009) reported that, during the first 48 h of infection, more than half of the nematodes died and all had hyphae piercing through the cuticle of the nematodes. This was followed by a second phase of slower killing by *C. albicans* without the production of hyphae. We therefore speculated that supplementation with EPA, may influence the ability of *C. albicans* to form hyphae and initiate this first rapid killing phase of the infection. Upon closer inspection, it was seen that, during the first 2 days there was hyphal production in AA supplemented nematodes infected with *C. albicans* SC5314 (although less than in unsupplemented nematodes), and thus rapid death of nematodes (Figure 3.4C). This was followed by the slower killing phase with the absence of hyphae, similar to results of unsupplemented nematodes infected with *C. albicans* SC5314 (Figure 3.4B). Strikingly, no hyphal formation was observed in EPA supplemented nematodes infected with *C. albicans* SC5314 (Figure 3.4D), indicating that EPA supplementation either affects hyphal production

by *C. albicans* directly or influences *C. elegans* via as yet unknown mechanism to indirectly inhibit hyphal formation by the yeast.

In *C. albicans*, the switching of yeast cells to hyphal cells is considered to play an important role in biofilm formation and the pathogenesis of fungal infections (Chandra *et al*., 2001; Douglas, 2003). Recently, several medium chain FAs were reported to inhibit hyphal growth in *C. albicans* (Lee *et al*., 2020; Kim *et al*., 2021). Kim and co-workers (2021) discovered that saw palmetto oil, lauric acid (12:0) and myristic acid (14:0) substantially suppressed the hyphal transition, while untreated control cells were predominantly hyphal cells. Moreover, Lee and co-workers (2020) reported that 12:0 inhibited *C. albicans* biofilm formation by down-regulation of several hyphal and biofilm-related genes. Also, it was reported that 14:0 inhibited biofilm and hyphal formation by *C. albicans*, probably targeting several proteins involved in ergosterol synthesis, sphingolipid metabolism, multidrug resistance and the oxidative stress (Prasath *et al*., 2019). As expected, *C. elegans* survived well with *E. coli* OP50, which is a common food source for the nematode, while the lifespan of *C. elegans* was much decreased in the presence of *C. albicans* (Kim *et al*., 2021). However, saw palmetto oil, 12:0 and 14:0 were found to markedly prolong *C. elegans* survival in the presence of *C. albicans* (Kim *et al*., 2021).

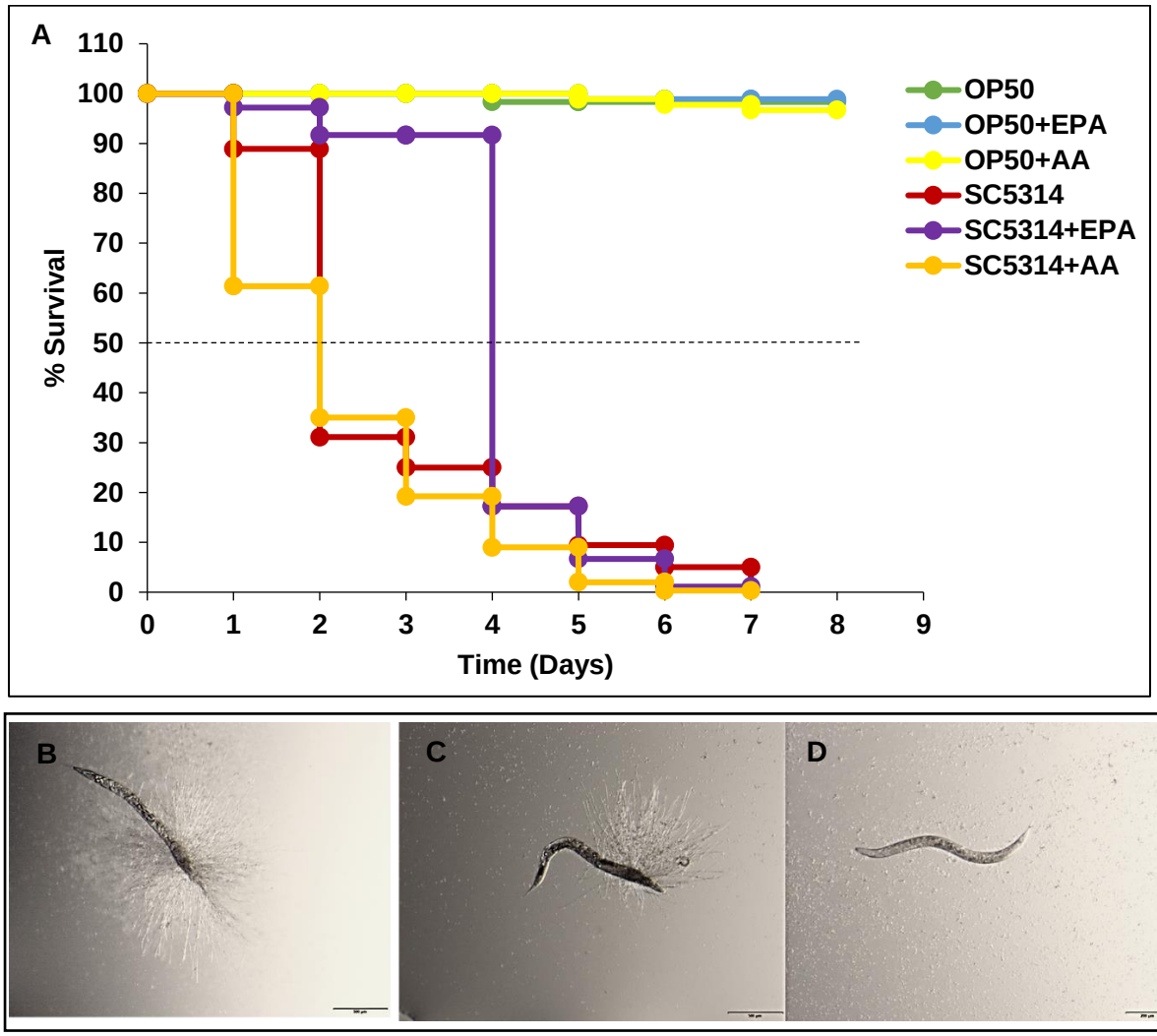


Figure 3.4. (A) *Candida albicans* killing of *Caenorhabditis elegans* supplemented with fatty acid (FA) sodium salts. The survival of nematodes with [arachidonic acid (AA) or eicosapentanoic acid (EPA)] or without FAs were significantly reduced when exposed to *C. albicans* SC5314 compared to *Escherichia coli* OP50 control ($P < 0.05$). Unsupplemented nematodes are more susceptible to killing by *C. albicans* ($P < 0.01$). The EPA supplementation significantly rescued the initial susceptibility to pathogen within 4 days of infection. Significance was determined using Kaplan-Meier survival curves and log-rank tests. Statistical analyses performed using two-way ANOVA with Bonferroni correction ($P < 0.05$). Dotted line represents 50% killing of *C. elegans*. (B) *C. albicans* SC5314 infected nematodes with hyphal production. (C) AA supplemented *C. albicans* SC5314 infected nematodes with hyphal production. (D) EPA supplemented *C. albicans* SC5314 infected nematodes with no hyphal production. Scale bars represent 200 µm.

Table 3.2. Effect of *C. albicans* on lifespan of fatty acid supplemented nematodes *C. elegans.*

Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
Unsupplemented nematodes with *E. coli* OP50	6.94	0.03	-	-
AA supplemented nematodes with *E. coli* OP50	6.67	0.07	-	0.0003[a]
EPA supplemented nematodes with *E. coli* OP50	6.98	0.02	-	1.000[a]
Unsupplemented nematodes with *C. albicans* SC5314	2.79	0.12	2.00	< 0.001[a]
AA supplemented nematodes with *C. albicans* SC5314	3.07	0.17	3.00	< 0.001[a]; 1.000[b]
EPA supplemented nematodes with *C. albicans* SC5314	4.02	0.07	4.00	< 0.001[a]; < 0.001[b]

[a] comparison to unsupplemented *E. coli* OP50, [b] comparison to unsupplemented *C. albicans* SC5314.

4.4.2. Fatty acids modulate the survival of bacterially infected nematodes

We analysed the effect of supplementation with AA and EPA on *P. aeruginosa* PAO1 infected nematodes. Results show that 50% of unsupplemented nematodes, infected by *P. aeruginosa* died within 2 days and 100% died within 6 days (Figure 3.5 and Table 3.2). Although EPA supplementation seemed to enhance survival of *C. elegans* infected with *C. albicans* (Figure 3.4A), this was not the case for nematodes infected with *P. aeruginosa* PAO1 (Figure 3.5). In fact, EPA supplemented nematodes infected with *P. aeruginosa* were more susceptible and had 100% mortality within 5 days (Figure 3.5), compared to 6 days for unsupplemented and AA-supplemented nematodes. Supplementation of AA significantly extended the time needed to kill 50% of the nematodes to 3 days, although it did not influence the time needed to kill 100% of the nematodes compared to unsupplemented *P. aeruginosa* infected nematodes (Figure 3.5). Overall, the dietary supplementation of either AA or EPA to nematodes did have

an influence on nematodes susceptibility to *P. aeruginosa*, confirming that this 20-carbon PUFAs do have detectable roles in susceptibility to this pathogen.

Previous studies demonstrated that FA supplementation have diverse roles in pathogenic bacterial susceptibility (Nandakumar and Tan, 2008; Anderson *et al*., 2019). For instance, using the *C. elegans-P. aeruginosa* host-pathogen model, it is demonstrated that Δ6 desaturase is required for basal innate immunity *in vivo*, through 18:3n-6 and stearidonic acid (18:4n-3), but not AA and EPA (Nandakumar and Tan, 2008). Furthermore, evidence supported the hypothesis that deficiencies in 18:3n-6 and 18:4n-3 result in increased susceptibility to *P. aeruginosa* bacterial infection, which is linked to reduced basal expression of numerous immune-specific genes encoding antimicrobial peptides (Nandakumar and Tan, 2008). Therefore, this renders 18:3n-6 and 18:4n-3 vital in maintaining basal activity of the p38 mitogen-activated protein kinase pathway, which functions in protecting most metazoan animals from various infections, and also from oxidative stress (Nandakumar and Tan, 2008). Recently, Anderson and co-workers (2019), demonstrated that oleate is essential for the activation of innate immune and resistance to bacterial infection in a distinctive manner compared to the effects of 18:3n-6 and 18:4n-3. For instance, exogenous oleate was unable to rescue the enhanced susceptibility of the *fat-3(wa22)* mutant to pseudomonal infection (Anderson *et al*., 2019). Moreover, the *fat-6* and *fat-7* seemed to have an effect on pathogen susceptibility through oleate production, however independent of PUFA biosynthesis via *fat-2* or *fat-3* enzymes (Anderson *et al*., 2019). In *Staphylococcus aureus*, several FAs such as 16:0, 18:1n-9, 18:2n-6, eicosadienoic acids (20:2n-6), 22:6n-3 and EPA at sub-minimum inhibitory concentration levels, inhibited biofilm formation and haemolytic activity in *S. aureus* (Kim *et al*., 2018; Kumar *et al*., 2020). Moreover, Kim *et al.* (2021) discovered that palmetto oil, 12:0 and 14:0 reduced virulence of *S. aureus* in *C. elegans* and exhibited minimal cytotoxicity.

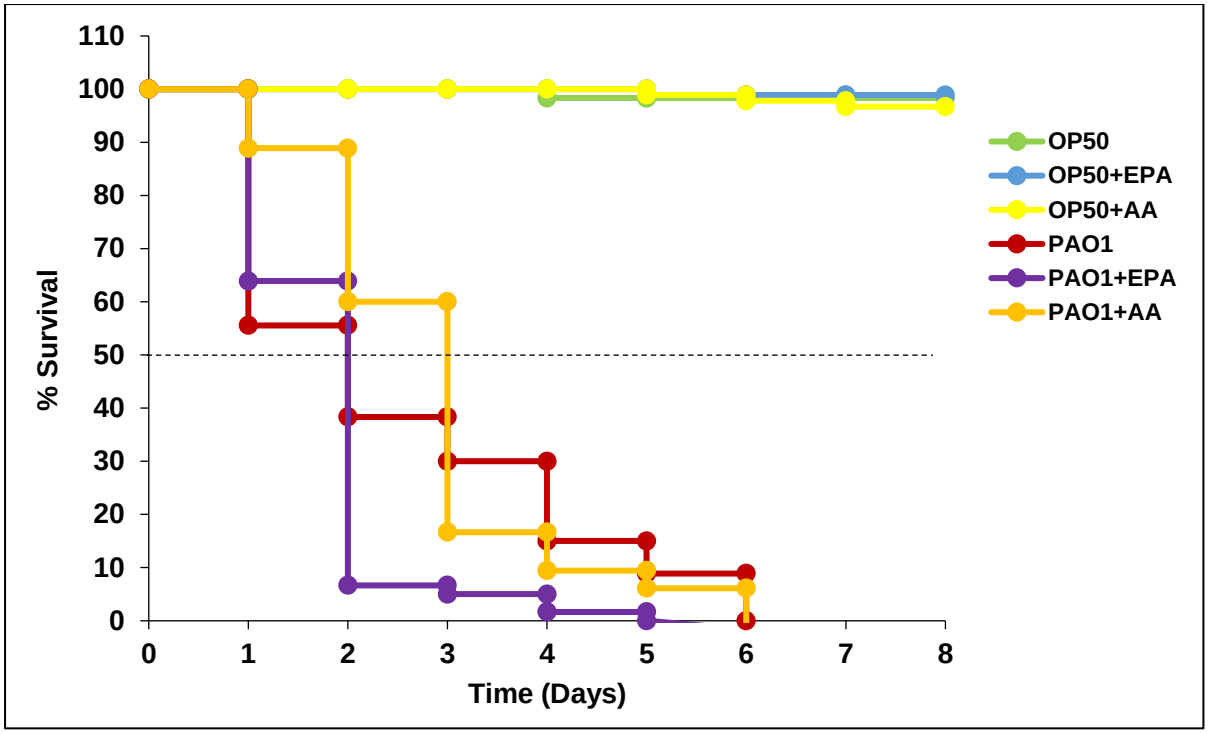


Figure 3.5. *Pseudomonas aeruginosa* killing of *Caenorhabditis elegans* supplemented with fatty acid (FA) sodium salts. The lifespan of *C. elegans* is decreased significantly in the presence of *P. aeruginosa* PAO1 compared to *Escherichia coli* OP50. The FAs supplementation had a significant effect on the survival of infected nematodes. Significance was determined using Kaplan-Meier survival curves and log-rank tests. Statistical analyses performed using two-way ANOVA with Bonferroni correction. Dotted line represents 50% killing of *C. elegans.*

Table 3.3. Effect of *P. aeruginosa* on lifespan of fatty acid supplemented nematodes *C. elegans.*

Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
Unsupplemented nematodes with *E. coli* OP50	6.94	-	-	-
AA supplemented nematodes with *E. coli* OP50	6.67	-	-	< 0.001[a]
EPA supplemented nematodes with *E. coli* OP50	6.98	-	-	1.000[a]

Unsupplemented nematodes with *P. aeruginosa* PAO1	2.48	0.13	2.00	< 0.001[a]
AA supplemented nematodes with *P. aeruginosa* PAO1	2.66	0.08	3.00	< 0.0001[a]; 1.000[b]
EPA supplemented nematodes with *P. aeruginosa* PAO1	1.72	0.05	2.00	< 0.001[a]; < 0.001[b]

[a] comparison to unsupplemented nematodes with *E. coli* OP50, [b] comparison to unsupplemented nematodes with *P. aeruginosa* PAO1.

4.4.3. Fatty acids modulate co-infection of infected nematodes survival

Lastly, we determined if AA or EPA affects susceptibility in *C*. *elegans* infected with both *C. albicans* SC5314 and *P*. *aeruginosa* PAO1. Similar to *P. aeruginosa* infections of unsupplemented nematodes, we observed that supplementation with EPA significantly reduced the survival of nematodes infected with both *P. aeruginosa* and *C. albicans* (Figure 3.6). Interestingly, in this case AA-supplementation increased the total time required to kill 100% of the nematodes infected by both pathogens to 6 days, compared to 5 days in the case of unsupplemented and EPA-supplemented nematodes. This increased survival was not observed in AA-supplemented nematodes infected only with *P. aeruginosa* (Figure 3.5). Consistent with previous report, no hyphal production was observed during the co-infection of the nematodes with and without FA sodium salt supplementations. Noteworthy, at present FAs and FAs salts are known to exhibit antibacterial effects on oral bacteria (Huang *et al*., 2011; Hara *et al*., 2021). Moreover, oral bacteria in a biofilm are also known to produce FAs that act as endogenous antibacterial agents, inhibiting the growth of other species in the biofilm and suppressing plaque formation (Hayes, 1984; Hojo, 2009). However, the mechanism behind antimicrobial activity of FA salts is still unclear.

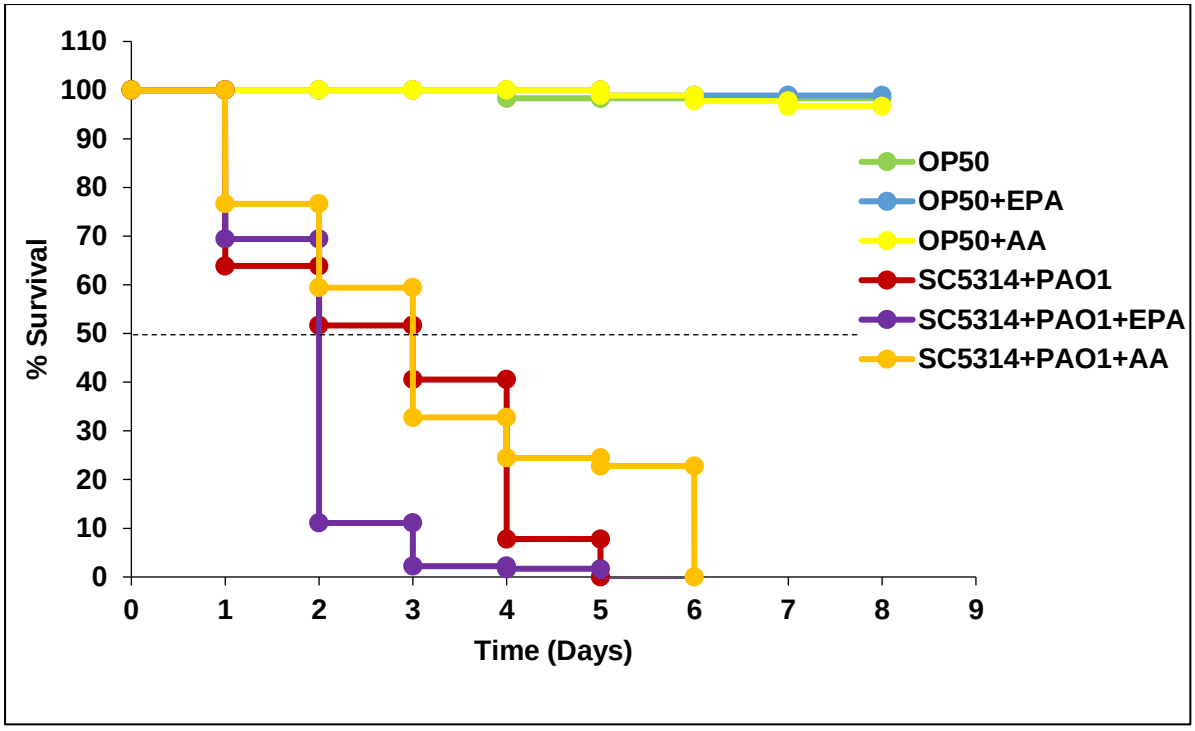


Figure 3.6. *Candida albicans* and *Pseudomonas aeruginosa* co-infection killing of fatty acid supplemented nematodes over time. Significance was determined using Kaplan-Meier survival curves and log-rank tests. Statistical analyses performed using two-way ANOVA with Bonferroni correction. Dotted line represents 50% killing of *C. elegans*.

Table 3.4. Effect of *C. albicans* and *P. aeruginosa* co-infection on lifespan of fatty acid supplemented *C. elegans.*

Conditions	Median lifespan (Days)	Standard error (S. E.)	Days to reach 50% survival	Log-rank test *P*-value (Bonferroni *P*-value)
Unsupplemented nematodes with *E. coli* OP50	6.94	-	-	-
AA supplemented nematodes with *E. coli* OP50	3.16	-	-	0.0003[a]
EPA supplemented nematodes with *E. coli* OP50	5.99	-	-	1.000[a]

Unsupplemented nematodes with *C. albicans* SC5314 and *P. aeruginosa* PAO1	2.64	0.11	2.00	< 0.001[a]
AA supplemented nematodes with *C. albicans* SC5314 and *P. aeruginosa* PAO1	1.84	0.05	3.00	< 0.001[a]; 1.000[b]
EPA supplemented nematodes with *C. albicans* SC5314 and *P. aeruginosa* PAO1	2.40	0.10	2.00	< 0.001[a]; < 0.001[b]

[a] comparison to unsupplemented *E. coli* OP50, [b] comparison to unsupplemented *C. albicans* SC5314 and *P. aeruginosa* PAO1.

4.5. Influence of PUFA supplementation on egg retention

The purpose of this study was to better understand the influence of PUFAs on the response of nematodes to stress in a *C. albicans-P. aeruginosa-C. elegans* infectious model system. As was also seen in chapter 2, exposure to pathogens, causes a change in egg retention behaviour, which depended on the specific pathogen present and was most pronounced when the nematodes were exposed to both *C. albicans* and *P. aeruginosa.* Moreover, the observed high egg retention seen in the unsupplemented co-infection, correlates with the increased virulence of the unsupplemented co-infection observed in the liquid killing assay (Figure 3.7). Furthermore, consistent with these results, we observed an effect of PUFAs on the egg retention of the infected nematodes. Although there seems to be an increase in egg retention of both the uninfected and infected AA supplemented nematodes, it was not significantly different compared to unsupplemented uninfected and infected nematodes (Figure 3.7). This pattern suggested that these AA supplemented nematodes were unable to lay eggs efficiently despite the AA supplementation, thus suggesting that AA might not have a significant role in egg retention of infected nematodes. Interestingly, a more striking effect of the EPA is that there was a significant decrease in the egg retention of EPA supplemented nematodes with *C. albicans* SC5314 ($P = 0.0007$), *P. aeruginosa* PAO1 ($P = 0.0002$) and combined infection ($P = 0.0047$), compared to unsupplemented infected nematodes (Figure 3.7). Thus, revealing that the EPA may have a significant effect on the egg retention of *C. elegans* and pathogenesis of *C. albicans* and *P. aeruginosa.* Also, this proves that EPA does have an effect on egg laying of nematodes especially in the presence of pathogens. Taking into consideration that research shows that the number of eggs in the uterus is a function of both the rate of egg production and the rate of egg laying (Schafer, 2005; Gardner *et al*., 2013). It can also be speculated that

this varied effect may be due to the expression of different virulence factors in each strain. The exact mechanism by which these pathogens affect nematode egg retention requires further studies.

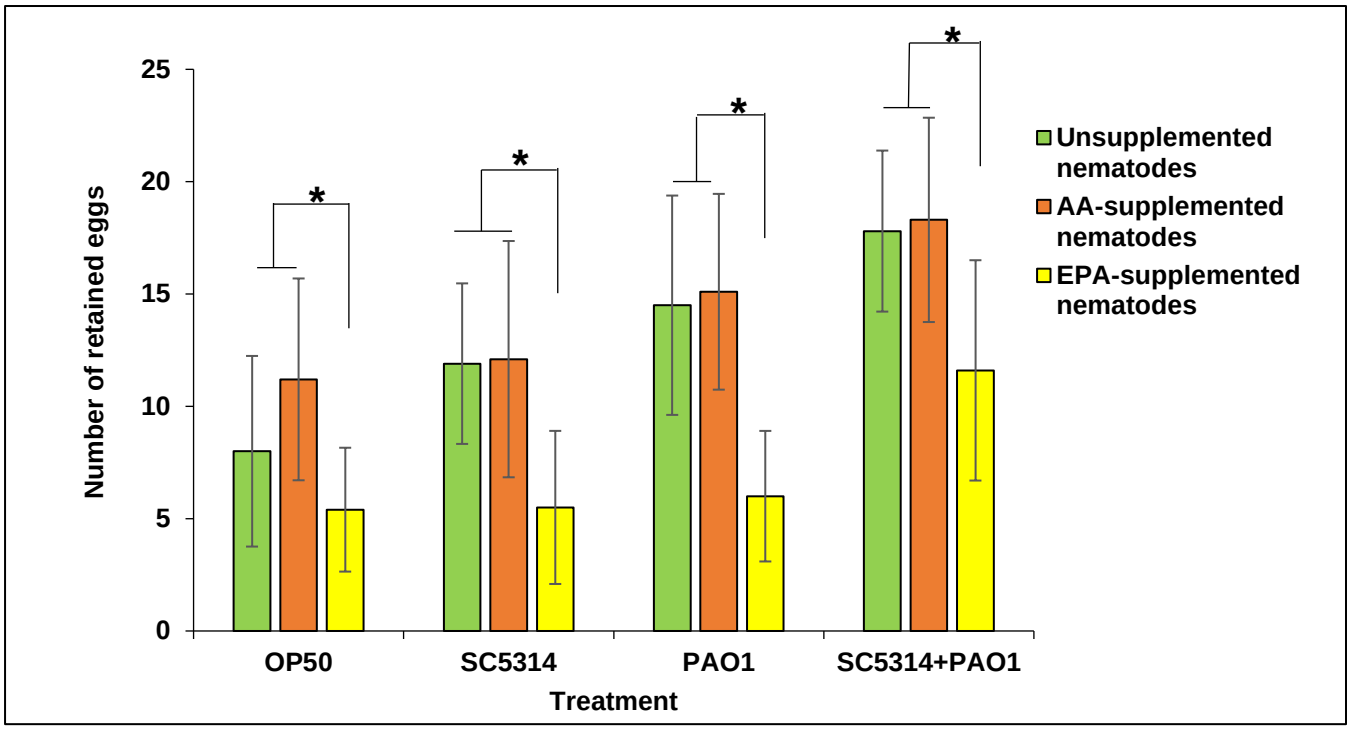


Figure 3.7. Egg-in-worm assay of fatty acid supplemented *Caenorhabditis elegans* infected with *Candida albicans* and *Pseudomonas aeruginosa*. Nematodes exposed to either or both *C. albicans* SC5314 and *P. aeruginosa* PAO1 showed an increased level of egg retention compared to nematodes fed *Escherichia coli* OP50 control strain. While arachidonic acid (AA) supplemented nematodes infected with either or both pathogens showed an increased level of egg retention, eicosapentanoic acid (EPA) supplemented infected nematodes showed a decreased egg retention. Each experiment was performed on triplicate on different days on ten nematodes each. Asterisk (*) indicate significant difference ($P < 0.05$; Student's *t*-test).

Noteworthy, the functions of n-3 and n-6 PUFAs in oocytes and embryo development of *C. elegans* are still under debate. It is likely that PUFAs are required for multiple processes to ensure optimum reproductive output. One process involves signalling molecules derived from PUFAs that are required in the female germ line for sperm guidance toward oocytes (Figure 3.8) (Kubagawa *et al*., 2006; Watts and Browse, 2006; Edmonds *et al*., 2010; Hoang *et al*., 2013). Follow up studies showed that these PUFAs, including AA and EPA, are synthesized in the intestine and transported to oocytes to be converted into F-series prostaglandins to

guide the sperm under the regulation of the insulin signalling in the intestine and TGF-β pathway in sensory neurons, thus to completely control the output of reproduction (Kubagawa *et al*., 2006; Edmonds *et al*., 2010; Hoang *et al*., 2013; McKnight *et al*., 2014). Fascinatedly, both signalling pathways respond when conditions are favourable such as during food availability. However, during food scarcity, there is reduction in the biosynthesis of prostaglandin, thus leading to reduced effectiveness of sperm localizing the fertilization site, eventually decreasing the rate of fertilization. Moreover, when wild-type males were mated to *fat-2* mutants, which are unable to synthesize PUFAs, the sperm failed to migrate toward the spermatheca, the region of the uterus where fertilization occurs (Kubagawa *et al*., 2006). However, when *fat-2* mutants were provided dietary 20-carbon PUFAs, either n-3 or n-6 species, sperm migration greatly improved (Kubagawa *et al*., 2006). Therefore, in this study, it is likely that the observed release of eggs of infected and uninfected nematodes supplemented with EPA were influenced by this process, thus promoting sperm movement to the fertilization site, eventually increasing the rate of fertilisation and release of eggs from the nematodes.

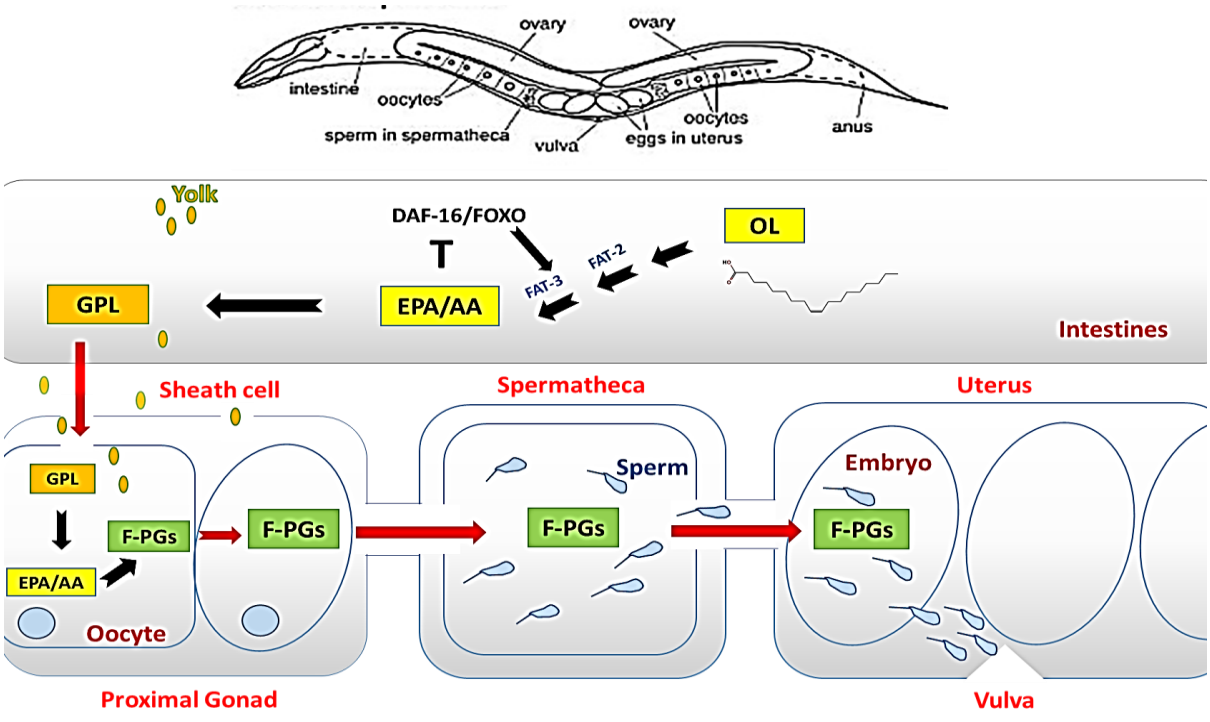


Figure 3.8. Guidance of sperm to the in spermatheca for egg fertilization in *Caenorhabditis elegans* by eicosanoids, F-PGs. In the intestines, DAF-16/FOXO promotes biosynthesis of PUFA (AA or EPA) through increased fat-family genes expression. The PUFAs regulates DAF-16 negatively to promote oocyte yolk endocytosis and PG synthesis. Then GPL-containing yolk translocates to oocytes in order to synthesize F-PGs that facilitates in sperm guidance.

Intercellular transportation of lipid molecules such as GPLs and F-PGs are indicated by red arrows. Abbreviations: AA, arachidonic acid; EPA, eicosapentaenoic acid; F-PGs, F-series prostaglandins; GPL, glycerophospholipid; OL, oleic acid (Mokoena *et al*., 2020).

---

5. Conclusions

Caenorhabditis elegans is one of the adaptable model organisms which offers many experimentally desirable traits. Within this model system, we were successfully able to address important questions that may lead to alternative solution to finding potential antimicrobial for polymicrobial infections. In summary, our results demonstrate that 20-carbon PUFAs, AA and EPA, appear to have minimal effects on the infection response to *C. albicans* and *P. aeruginosa* clinical isolates. In particular, supplementation with EPA resulted in a partially reduced *C. albicans* virulence in a nematode infection model. Moreover, our observation of EPA supplementation inhibiting hyphal production of *C. albicans* suggests that EPA might in particular have altering function that is required for inhibition of yeast to hyphal transition. The identification of these EPA targets might lead to the discovery of additional inhibitors of fungal morphogenesis with broader application. Moreover, non-toxic molecules such as FAs that are able to inhibit yeast to hyphal conversion and hyphal growth of *C. albicans* might result in us understanding the pathogenic fungal morphogenesis and may serve as templates for the development of novel antifungal agents. In addition, guided by the previously observed link between altered FA profiles and infections, we sought to investigate the potential of PUFAs to act as an antimicrobial agent in conjunction. We observed that despite these alterations in FA composition, survival on these supplements was not sufficient to rescue the nematodes from infection. Although our results from the gas chromatography analysis indicates that AA and EPA have significant effect on the FA profiles of both the mono- and polymicrobial infections of *C. elegans* by bacterial and fungal pathogens, but not on survival, we cannot not rule out other, as yet unidentified, roles for these 20-carbon FAs in the pathogenesis. Therefore, to address the functions of these molecules, we will need to generate *C. elegans* mutants depleted of LC-PUFAs and analysed their functions. This system could be used as a basis for the development of novel antimicrobial approaches to prevent microbial infections. Moreover, this research will hopefully shed some light on the growing area of research of interkingdom communication.

6. References

Anderson, S. M., Cheesman, H. K., Peterson, N. D., Salisbury, J. E., Soukas, A. A. & Pukkila-Worley, R. (2019). The fatty acid oleate is required for innate immune activation and pathogen defense in *Caenorhabditis elegans*. *PLoS Pathogens* **15** (6).

Bergsson, G., Steingrímsson, Ó. & Thormar, H. (1999). *In vitro* susceptibilities of *Neisseria gonorrhoeae* to fatty acids and monoglycerides. *Antimicrob Agents Chemother* **43**, 2790–2792.

Bodhicharla, R., Devkota, R., Ruiz, M. & Pilon, M. (2018). Membrane fluidity is regulated cell nonautonomously by *Caenorhabditis elegans* PAQR-2 and its mammalian homolog AdipoR2. *Genetics* **210**, 189–201.

Bouyanfif, A. (2019). Effects of fatty acid supplementation on gene expression, lifespan, and biochemical changes in wild type and mutant *C. elegans* strains. Texas Tech University, PhD Dissertation. https://hdl.handle.net/2346/85937

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M. & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog* **3**, e18.

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

Brock, T. J., Browse, J. & Watts, J. L. (2006). Genetic regulation of unsaturated fatty acid composition in *C. elegans*. *PLoS Genet* **2**, e108.

Caron, E., Desseyn, J. L., Sergent, L., Bartke, N., Husson, M. O., Duhamel, A. & Gottrand, F. (2015). Impact of fish oils on the outcomes of a mouse model of acute *Pseudomonas aeruginosa* pulmonary infection. *Br J Nutr* **7**, 1–9.

Chandra, J., Kuhn, D. M., Mukherjee, P. K., Hoyer, L. L., McCormick, T. & Ghannoum, M. A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: Development, architecture, and drug resistance. *J Bacteriol* **183**, 5385-5394.

Clement, M., Tremblay, J., Lange, M., Thibodeau, J. & Belhumeur, P. (2007). Whey-derived free fatty acids suppress the germination of *Candida albicans in vitro*. *FEMS Yeast Res* **7**, 276-285.

Correia, M., Miche, L. V., Matos, A. A., Carvalho, P., Oliveira, M. J., Ferreira, R. M., Dillies, M. A., Huerre, M. *et al*. (2012). Docosahexaenoic acid inhibits *Helicobacter pylori* growth *in vitro* and mice gastric mucosa colonization. *PLoS One* **7**, e35072.

Corsi, A. K., Wightman, B. & Chalfie, M. (2015). A transparent window into biology: A primer on *Caenorhabditis elegans*. *Genetics* **200**, 387–407.

Cui, C., Song, S., Yang, C., Sun, X., Huang, Y., Li, K. *et al*. (2019). Disruption of quorum sensing and virulence in *Burkholderia cenocepacia* by a structural analogue of the *cis*-2-dodecenoic acid signal. *Appl Environ Microbiol* **85**, e00105-00119.

Davies, D. G. & Marques, C. N. H. (2009). A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *J Bacteriol* **191**, 1393– 1403.

Deline, M. L., Vrablik, T. L. & Watts, J. L. (2013). Dietary supplementation of polyunsaturated fatty acids in *Caenorhabditis elegans*. *J Vis Exp* **81**, e50879.

Desbois, A. & Smith, V. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol* **85**, 1629-1642.

Douglas, L. J. (2003). *Candida* biofilms and their role in infection. *Trends Microbiol* **11**, 30–36.

Diaz, M. T., Alvarez, I., De La Fuente, J., Sanudo, C., Campo, M. M., Oliver, M. A., Fonti Furnols, M., Montossi, F., San Julian, R., Nute, G. R. & Caneque, V. (2005). Fatty acid composition of meat from typical lamb production systems of Spain, United Kingdom, Germany and Uruguay. *Meat Science* **71**, 256-263.

Edmonds, J. W., Prasain, J. K., Dorand, D., Yang, Y., Hoang, H. D., Vibbert, J., Kubagawa, H. M. & Miller, M. A. (2010). Insulin/FOXO signaling regulates ovarian prostaglandins critical for reproduction. *Dev Cell* **19**, 858–871.

Estes, K. A., Dunbar, T. L., Powell, J. R., Ausubel, F. M. & Troemel, E. R. (2010). bZIP transcription factor *zip-2* mediates an early response to *Pseudomonas aeruginosa* infection in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **107**, 2153–2158.

Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**, 497-509.

Gardner, M., Rosell, M. & Myers, E. M. (2013). Measuring the effects of bacteria on *C. elegans* behavior using an egg retention assay. *J Vis Exp* **80**, e51203.

Han, S. K., Lee, D., Lee, H., Kim, D., Son, H. G. *et al*. (2016). OASIS 2: online application for survival analysis 2 with features for the analysis of maximal lifespan and health span in aging research. *Oncotarget* **7**, 56147–56152.

Han, S., Schroeder, E. A., Silva-García, C. G., Hebestreit, K., Mair, W. B. & Brunet, A. (2017). Mono-unsaturated fatty acids link H3K4me3 modifiers to *C. elegans* lifespan. *Nature* **544**, (7649), 185–190.

Hara, T., Sonoi, A., Handa, T., Okamoto, M., Kaneko, E., Ikeda, R., Habe, T., Fujinaka, H., Inoue, S. & Ichikawa, T. (2021). Unsaturated fatty acid salts remove biofilms on dentures. *Sci Rep* **11** (1), 12524.

Hayes, M. L. (1984). The effects of fatty acids and their monoesters on the metabolic activity of dental plaque. *J Dent Res* **63**, 2–5.

Hoang, H. D., Prasain, J. K., Dorand, D. & Miller, M. A. (2013). A heterogeneous mixture of F-series prostaglandins promotes sperm guidance in the *Caenorhabditis elegans* reproductive tract. *PLoS Genet* **9**, 19.

Hojo, K., Nagaoka, S., Ohshima, T. & Maeda, N. (2009). Bacterial interactions in dental bioflm development. *J Dent Res* **88**, 982–990.

Hou, N. S., Gutschmidt, A., Choi, D. Y., Pather, K., Shi, X. *et al*. (2014). Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis *in vivo*. *Proc Natl Acad Sci USA* **111**, E2271–2280.

Huang, C. B., Altimova, Y., Myers, T. M. & Ebersole, J. L. (2011). Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Arch Oral Biol* **56**, 650–654.

Hur, S. J., Ye, B. W., Lee, J. L., Ha. Y. L., Park, G. B. & Joo, S. T. (2004). Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. *Meat Science* **66**, 771-775.

Inoue, T., Shingaki, R. & Fukui, K. (2008). Inhibition of swarming motility of *Pseudomonas aeruginosa* by branched-chain fatty acids. *FEMS Microbiol Lett* **281**, 81– 86.

Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis Rep* **48**, 692.

Kim, Y. -G., Lee, J. -H., Park, S., Kim, S. & Lee, J. (2021). Inhibition of polymicrobial biofilm formation by saw palmetto oil, lauric acid and myristic acid. *Microb Biotechnol* **00** (00), 1– 13

Kim, Y. -G., Lee, J. -H., Raorane, C. J., Oh, S. T., Park, J. G. & Lee, J. (2018). Herring oil and omega fatty acids inhibit *Staphylococcus aureus* biofilm formation and virulence. *Front Microbiol* **9**, 1241.

Kubagawa, H. M., Watts, J. L., Corrigan, C., Edmonds, J. W., Sztul, E., Browse, J. & Miller, M. A. (2006). Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment *in vivo*. *Nat Cell Biol* **8**, 1143–1148.

Kumar, P., Lee, J. H., Beyenal, H. & Lee, J. (2020). Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol* **28**, 753– 768.

Lee, J. -H., Kim, Y. -G., Khadke, S. K. & Lee, J. (2020). Antibiofilm and antifungal activities of medium-chain fatty acids against *Candida albicans* via mimicking of the quorum-sensing molecule farnesol. *Microb Biotechnol* **14**, 1353–1366.

Margie, O., Palmer, C. & Chin-Sang, I. (2013). *C. elegans* chemotaxis assay. *J Vis Exp* **74**, e50069.

McKnight, K., Hoang, H. D., Prasain, J. K., Brown, N., Vibbert, J. *et al*. (2014). Neurosensory perception of environmental cues modulates sperm motility critical for fertilization. *Science* **344**, 754–757.

McLain, N., Ascanio, R., Baker, C., Strohaver, R. A. & Dolan, J. W. (2000). Undecylenic acid inhibits morphogenesis of *Candida albicans*. *Antimicrob Agents Chemother* **44**, 2873–2875.

Mil-Homens, D., Rocha, E. P. C. & Fialho, A. M. (2010). Genome-wide analysis of DNA repeats in *Burkholderia cenocepacia* J2315 identifies a novel adhesin-like gene unique to epidemic-associated strains of the ET-12 lineage. *Microbiology* **156**, 1084–1096.

Mokoena, N. Z., Sebolai, O. M., Albertyn, J. & Pohl, C. H. (2020). Synthesis and function of fatty acids and oxylipins, with a focus on *Caenorhabditis elegans. Prostag Oth Lipid M* **148**, 106426.

Murzyn, A., Krasowska, A., Stefanowicz, P., Dziadkowiec, D. & Lukaszewicz, M. (2010). Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. *PLoS One* **5**, e12050.

Muthamil, S., Balasubramaniam, B., Balamurugan, K., & Pandian, S. K. (2018). Synergistic effect of quinic acid derived from syzygium cumini and undecanoic acid against *Candida* spp. biofilm and virulence. *Front Microbiol* **9**, 2835.

Nandakumar, M. & Tan, M. W. (2008). Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity. *PLoS Genet* **4**, e1000273.

Noverr, M. C. & Huffnagle, G. B. (2004). Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. *Infect Immun* **72**, 6206–6210.

Papsdorf, K. & Brunet, A. (2018). Linking lipid metabolism to chromatin regulation in aging. *Trends in Cell Biol* **1461**, 1-20.

Prasath, K. G., Sethupathy, S. & Pandian, S. K. (2019). Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. *J Proteomics* **208**, 103503.

Pukkila-Worley, R., Peleg, A. Y., Tampakakis, E. & Mylonakis, E. (2009). *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryot Cell* **11**, 1750-1758.

Qi, W., Gutierrez, G. E., Gao, X., Dixon, H., Joe A., McDonough, J. A., Marini. A. M. & Fisher, A. L. (2017). The ω-3 fatty acid α-linolenic acid extends *Caenorhabditis elegans* lifespan via NHR-49/PPARα and oxidation to oxylipins. *Aging Cell* **16**, 1125–1135.

Ramanathan, S., Ravindran, D., Arunachalam, K. & Arumugam, V. R. (2018). Inhibition of quorum sensing-dependent biofilm and virulence genes expression in environmental pathogen *Serratia marcescens* by petroselinic acid. *Antonie Van Leeuwenhoek* **111**, 501–515.

Ruxton, C. H., Reed, S. C., Simpson, M. J. & Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet* **17**, 449–459.

Santhakumari, S., Nilofernisha, N. M., Ponraj, J. G., Pandian, S. K. & Ravi, A. V. (2017). *In vitro* and *in vivo* exploration of palmitic acid from *Synechococcus elongatus* as an antibiofilm agent on the survival of *Artemia franciscana* against virulent vibrios. *J Invertebr Pathol* **150**, 21–31.

Schafer, W. R. (2005). Egg-laying, *WormBook*, ed. The *C. elegans* Research Community, WormBook.

Shin, S. Y., Bajpai, V. K., Kim, H. R. & Kang, S. C. (2007). Antibacterial activity of bioconverted eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) against foodborne pathogenic bacteria. *Int J Food Microbiol* **113**, 233–236.

Slover, H. T. & lanza, E. (1979). Quantitative analysis of food fatty acid by capillary gas chromatography. *J Am Oil Chem Soc* **112**, 1032-1040.

Thibane, V. S., Ells, R., Hugo, A., Albertyn, J., van Rensburg, W. J., Van Wyk, P. W., Kock, J. L. & Pohl, C. H. (2012). Polyunsaturated fatty acids cause apoptosis in *C. albicans* and *C. dubliniensis* biofilms. *Biochim Biophys Acta* **1820**, 1463-1468.

Tiesset, H., Pierre, M., Desseyn, J. L., Guéry, B., Beermann, C., Galabert, C., Gottrand, F. & Husson, M. O. (2009). Dietary (n-3) polyunsaturated fatty acids affect the kinetics of pro- and anti-inflammatory responses in mice with *Pseudomonas aeruginosa* lung infection. *J Nutr* **139**, 82–89.

Watts, J. L. & Browse, J. (2006). Dietary manipulation implicates lipid signaling in the regulation of germ cell maintenance in *C. elegans*. *Dev Biol* **292**, 381–392.

Webster, C. M., Deline, M. L. & Watts, J. L. (2013). Stress response pathways protect germ cells from omega-6 polyunsaturated fatty acid-mediated toxicity in *Caenorhabditis elegans*. *Dev Biol* **373**, 14-25.

Wenderska, I. B., Chong, M., McNulty, J., Wright, G. D. & Burrows, L. L. (2011). Palmitoyl-$_{DL}$-carnitine is a multitarget inhibitor of *Pseudomonas aeruginosa* biofilm development. *Chem Bio Chem* **12**, 2759–2766.

Wicks, S. R., de Vries, C. J., van Luenen, H. G. & Plasterk, R. H. (2000). CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. *Dev Biol* **221**, 295–307.

Wood, W. B. (1987). The nematode *Caenorhabditis elegans*. W. B. Wood (Ed.), Cold Spring Harbor Laboratory Press, New York

Yang, Q., Alemany, R., Casas, J., Kitajka, K., Lanier, S. M. *et al*. (2005). Influence of the membrane lipid structure on signal processing via G protein-coupled receptors. *Mol Pharmacol* **68**, 210–217.

Chapter 4

Exploring the mechanism behind the effect of EPA supplementation on *C. albicans* infection

1. Abstract

The intake of omega-3 polyunsaturated fatty acids (n-3 PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is associated with manifold health benefits. However, the mechanism(s) of action and the molecular species or metabolite(s) responsible for the protective roles of the EPA in particular remain largely obscure. We examined how EPA could affect *Candida albicans* virulence in the *Caenorhabditis elegans* infection model, an alternative to mammalian host models. Our hypothesis is that one or more cytochrome P450 (CYP450) metabolise EPA to 17,18-epoxyeicosatetraenoic acid (17,18-EpETE; the most abundant CYP eicosanoid in *C. elegans*), which inhibits *C. albicans* hyphal formation. Therefore, to test the hypothesis, we treated *C. albicans* cells, both *in vitro* and *in vivo* using *C. elegans*, with 17,18-EpETE. It was observed that 17,18-EpETE could reduce hyphal formation *in vitro*. Strikingly, 17,18-EpETE supplemented nematodes contained only *C. albicans* yeast cells in the gut, suggesting that 17,18-EpETE treatment inhibited the yeast to hyphal conversion, thus inhibiting the formation of invasive hyphae by *C. albicans*. We further examined our hypothesis by testing if inhibitors of mammalian EPA-metabolizing CYP450s, 17-octadecynoic acid (17-ODYA) and 6-(2-propargyloxyphenyl)hexanoic acid (PPOH), could inhibit the *C. elegans* CYP450 activity and restore *C. albicans* hyphal formation. Indeed, the exogenously administered 17-ODYA and PPOH, significantly restored hyphal formation in *C. albicans* infected *C. elegans*. In order to gain a better understanding of the influence of EPA on the fatty acid (FA) metabolism and immune response of *C. elegans*, we studied the expression of *C. elegans* genes previously implicated in FA metabolism and immune response. Furthermore, to identify the effect of EPA on *C. albicans* hyphal formation in *C. elegans*, we also investigated genes involved in *C. albicans* hyphal formation. Using Nanostring (nCounter®), we observed differential expression of genes under the different conditions. This finding would provide the opportunity to use *C. elegans* as a model organism to facilitate the identification of the FAs with antimicrobial properties and the elucidation of signalling pathways mediating their physiological functions.

Key words: Eicosapentaenoic acid, *Candida albicans*, *Caenorhabditis elegans*, cytochrome P450, 17,18-epoxyeicosatetraenoic acid, 17-octadecynoic acid, 6-(2-propargyloxyphenyl)hexanoic acid, Nanostring

2. Introduction

Fatty acids (FAs) are not just important cellular structural components but are also important energy sources for mammals and have been suggested to be potential alternative antimicrobial agents (Desbois and Smith, 2010; Yoon *et al*., 2018). Recently, several studies reported that FAs exhibit anti-hyphal and antibiofilm activities at concentrations less than their minimum inhibitory concentrations (Kumar *et al*., 2020). For example, several FAs such as capric acid (10:0) and lauric acid (12:0) inhibit the growth of planktonic *Candida* cells (Bergsson *et al*., 2001), while butanoic acid (4:0), stearidonic acid (18:4n-3), eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (22:5n-6) and conjugated linoleic acid inhibit hyphal growth by *Candida albicans* (Noverr and Huffnagle, 2004; Murzyn *et al*., 2010; Shareck *et al*., 2011; Thibane *et al*., 2012; Muthamil *et al*., 2018; Prasath *et al*., 2019). *Candida albicans* has the ability to grow as either yeast or hyphae, and each form respectively contributes to its pathogenesis (Lo *et al*., 1997; Braun and Johnson, 1997; Cao *et al*., 2006). For instance, the yeast cells of *C. albicans* yeast can colonize mucosal surfaces thus facilitating the dissemination of the microrganism through the blood stream (Gow *et al*., 2002; Saville *et al*., 2003; Rosenbach *et al*., 2010). Hyphae, by contrast, are important for host invasion and tissue destruction (Lo *et al*., 1997; Berman and Sudbery, 2002; Saville *et al*., 2003; Kumamoto and Vinces, 2005). The factors that influence these diverse growth patterns during infection are poorly understood, but it is clear that innate immune mechanisms in mammalian epithelial cells normally prevent *C. albicans* from becoming a pathogen (Gantner *et al*., 2005; Moyes *et al*., 2010). Importantly, hyphal morphogenesis in *C. albicans* is tightly controlled by dozens of transcription factors that contribute to the activation or repression of the hyphal transcriptional program (Villa *et al*., 2020).

In our previous chapter, we showed that supplementation with EPA resulted in significant changes in FA profiles during infection of *C. elegans*. We also showed that EPA supplementation resulted in a partially reduced *C. albicans* virulence by influencing the early stages of infection in *C. elegans*. Moreover, EPA supplementation also inhibited hyphal formation of *C. albicans*. A new class of anti-allergic and anti-inflammatory lipid metabolite, 17,18-epoxyeicosatetraenoic acid (17,18-EpETE), synthesized from EPA through the enzymatic activity of cytochrome P450 (CYP450), has recently been identified in the control of allergic and inflammatory diseases in the skin and gut (Kunisawa *et al*., 2015; Serhan, 2017; Saika *et al*., 2019). However, the activity of EPA metabolite, 17,18-EpETE towards fungal infections has not been studied. In concent with this, our hypothesis is that major CYP450 epoxygenase isoforms preferentially metabolize EPA to 17,18-EpETE and that 17,18-EpETE plays a role in the inhibition of *C. albicans* hyphal formation (Figure 4.1). The current study

was undertaken to determine the effect of EPA on *C. albicans* hyphal formation and gene expression. To achieve this aim, we uncover the role of EPA in *C. albicans* infected *C. elegans*. We therefore wished to address the question of whether 17,18-EpETE alters the hyphal formation of *C. albicans* both *in vitro* and *in vivo* using *C. elegans*. We also determined the influence of EPA-metabolizing CYP450 enzymes inhibitors, 17-ODYA and PPOH. Nanostring (nCounter®) was used to confirm differential expression of selected genes in EPA supplemented and unsupplemented nematodes infected with *C. albicans*. We investigated the influence of EPA on *C. albicans* hyphal formation genes. We also used gene expression profiles of nematodes during an infection with *C. albicans* to define the antifungal immune response genes in the nematodes. Studies have also shown that many of the *C. albicans* genes required for virulence in murine models of infection are also required for virulence in nematodes (Nobile *et al*., 2008; Pukkila-Worley *et al*., 2009). For example, *RIM101*, a *C. albicans* transcription factor required for alkaline-induced hyphal growth, is required for both virulence in murine oropharyngeal candidiasis and virulence in *C. elegans* (Nobile *et al*., 2008; Pukkila-Worley *et al*., 2009). Other genes such as *NRG1*, *CAS5*, *ADA2*, *CPH1* and *EFG1* have also shown to be required for virulence in both mice and nematodes (Pukkila-Worley *et al*., 2009; Ford *et al*., 2015). These studies clearly demonstrate that many of the conclusions drawn using this simpler organism remain valid in higher mammals, reiterating the utility of *C. elegans* as a model host for the study of infectious diseases.

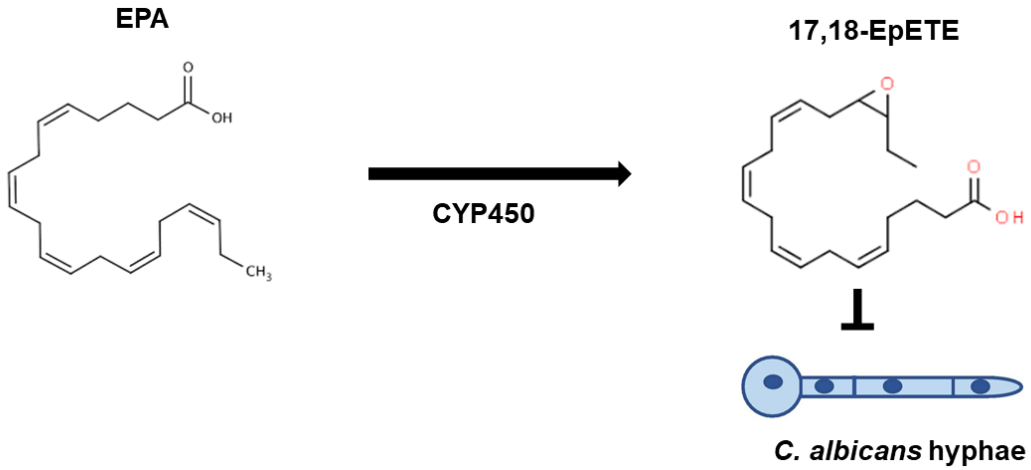


Figure 4.1. Hypothesis stating that cytochrome P450 (CYP450) activity metabolises eicosapentaenoic acid (EPA) to 17,18-epoxy eicosatetraenoic acid (17,18-EpETE), which inhibits *Candida albicans* hyphal formation.

3. Materials and methods

3.1. Strains used

Caenorhabditis elegans glp-4; sek-1 hermaphrodites, obtained from the *Caenorhabditis* Genetic Centre, College of Biological Sciences, University of Minnesota, were propagated on Nematode Growth Medium (NGM) (2.5 g/L peptone, 3 g/L sodium chloride, 17 g/L agar) spotted with *Escherichia coli* OP50 as food source, using the technique previously described by Brenner (1974). In all experiments, we used *E. coli* OP50 as our control. *Candida albicans* SC5314 was maintained on yeast extract-peptone-dextrose (YPD) agar (5 g/L peptone, 3 g/L yeast extract, 10 g/L glucose, 16 g/L agar) at 30 °C.

3.2. Influence of 17,18-epoxy eicosatetraenoic acid on germ tube formation *in vitro*

Candida albicans yeast cells were grown on YPD agar plates and incubated at 37 °C overnight. Nematode broth was prepared by growing *C. elegans* until larva 4 (L4) stage on *E. coli* OP50 seeded NGM agar plates. Thereafter, nematodes from 10 to 15 plates were carefully harvested and washed three times with sterile M9 buffer (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 5 g/L NaCl, 0.25 g/L $MgSO_4.7H_2O$). The nematode pellet was resuspended in 1.5 ml M9 Buffer, supplemented with glass beads (diameter 0.5 mm) and mechanically homogenized twice for 15 min using a Disruptor Genie Analog Cell Disruptor. The suspension was filtered using sterile syringe filter with a 0.2 µm pore size (GVS Filter Technology) to obtain nematode broth. Triplicate sets of test tubes containing either 5 ml of foetal bovine serum or nematode broth were inoculated with 2-3 colonies of *C. albicans*. Cell densities were adjusted to final cell concentration of approximately $l0^6$ cells/ml. The cell suspension was supplemented with 0.01 mM 17,18-epoxy eicosatetraenoic acid (17,18-EpETE) (Cayman Chemical) and incubated at 37 °C for 4 h. The cells were washed three times and suspended in 5 ml of phosphate-buffered saline (PBS, pH 6.8). All samples were transferred to an ice bath at the end of the incubation period prior to microscopic observation. A drop of each suspension was placed on microscope slides for examination of germ tubes. Percentage germ tubes formation was determined (Vediyappan *et al*., 2013).

3.3. Influence of 17,18-epoxy eicosatetraenoic acid on hyphal formation *in vivo*

To test the effect of 17,18-EpETE on *C. albicans* hyphal formation, a non-mammalian host model *C. elegans* was used as reported by Tampakakis *et al*. (2008) with slight modifications.

Briefly, synchronized larva 3 (L3) *C. elegans* nematodes that were grown for 24 h on *E. coli* OP50 seeded NGM agar plates with 17,18-EpETE added directly to the bacterial food at a final concentration of 0.01 mM. Plates were then incubated for 24 h for L3 staged nematodes to reach L4 stage. Thereafter nematodes were carefully harvested and washed three times with sterile M9 buffer. Synchronised L4 nematodes were infected with *C. albicans* according to the modified *C. elegans* liquid medium killing assay protocol by Breger *et al*. (2007). Subsequently, 60 to 70 nematodes were transferred into 2 ml of liquid medium (80% M9 buffer, 20% brain-heart infusion agar [(BHI, Sigma-Aldrich) (7.8 g/L brain extract, 9.7 g/L heart extract, 2.5 g/L disodium phosphate, 2.0 g/L dextrose, 15 g/L agar)] and 90 µg/ml kanamycin) in a single well of a six-well tissue culture plate and incubated at 25 °C for 24 h. Nematodes were monitored daily for hyphal formation.

3.4. Influence of CYP inhibitors on hyphal formation *in vivo*

Nematode growth medium agar with 10 ml 0.1% Tergitol (NP-40) (Sigma-Aldrich) was supplemented with 0.01 mM of EPA (Sigma-Aldrich). Synchronized L2 *C. elegans* nematodes were grown for 24 h on NGM agar plates with or without 0.01 mM EPA, seeded with *E. coli* OP50 until they reached L3 stage. Thereafter, the L3 nematodes were carefully harvested and washed three times with sterile M9 buffer. L3 nematodes were transferred to fresh plates and pre-treated for 24 h with 17-octadecynoic acid (17-ODYA) or 6-(2-propargyloxyphenyl)hexanoic acid (PPOH) (both from Cayman Chemicals) until they reached L4 stage. The compounds were added directly to the bacterial food at a final concentration of 0.05 mM (Kulas *et al*., 2008; Kosel *et al*., 2011). L4 nematodes were infected with *C. albicans* according to the modified *C. elegans* liquid medium killing assay protocol by Breger *et al*. (2007). Subsequently, 60 to 70 nematodes were transferred into 2 ml of liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) in a single well of a six-well tissue culture plate and incubated at 25 °C for 24 h. Nematodes were monitored daily for hyphal formation.

3.5. Fatty acid supplementation and infection of nematodes for gene expression studies

Nematode Growth Medium agar with 0.1% Tergitol (NP-40) was supplemented with 0.01 mM of EPA (Deline *et al*., 2013). Synchronized L4 *C. elegans* nematodes that were grown for 4 days on 60 NGM agar plates with or without 0.01 mM EPA, seeded with *E. coli* OP50, were carefully harvested and washed three times with sterile M9 buffer. Synchronised L4

nematodes were infected with *C. albicans* according to the modified *C. elegans* liquid medium killing assay protocol by Breger *et al*. (2007). Approximately 500 washed synchronized L4 *C. elegans* nematodes, grown on 60 NGM agar plates with or without 0.01 mM FA sodium salt seeded with *E. coli* OP50, were placed on the centre of a *C. albicans* lawn grown on BHI agar as well as the control nematodes then were incubated at 25 °C for 4 h. After incubation, nematodes were carefully washed off the BHI agar plates with 20 ml of sterile M9 buffer and transferred to 50 ml conical tube. Nematodes were thoroughly washed with M9 buffer four times. Any remaining microbial cells were removed via sucrose floatation during the washing step (Jenkins, 1964). Subsequently, the nematodes were transferred into sterile petri-dishes containing 10 ml liquid medium (80% M9 buffer, 20% BHI, 90 µg/ml kanamycin) and were incubated at 25 °C for 24 h. Following incubation, nematodes were washed by centrifugation at 4000 *g* for 2 min, the supernatant gently aspirated from pellet and 2 ml RNA*later* (Invitrogen) added to each sample to combat degradation of RNA. This was done in triplicate. Samples were frozen at -80 °C until RNA extraction.

3.6. Total RNA extraction

Samples were thawed on ice, centrifuged at 4000 *g* for 2 min to collect cells and RNA*later* was aspirated. The nematode pellet was resuspended in 600 µl of lysis buffer (Zymo Research), supplemented with 1 volume of glass beads (diameter 0.5 mm) and mechanically homogenized twice for 15 min using a Disruptor Genie Analog Cell Disruptor. Total RNA was extracted from samples using Quick-RNA MiniPrep kit (Zymo Research), including removal of the genomic DNA by DNase digestion, according to manufacturer's instructions. The RNA samples were evaluated using the Thermo Scientific NanoDrop ND-1000 Ultraviolet Visible Spectrophotometer to determine total RNA concentration in each sample.

3.7. Analyses of differential expression with nCounter®

Total RNA extracted from the different treatment conditions were analysed with the NanoString nCounter® analysis system (Geiss *et al*., 2008) at the University of the Witwatersrand, Department of Internal Medicine, using a gene expression TagSet that targets 123 *C. albicans* genes [including three housekeeping genes, *ACT1*, *LSC2*, *THD3* (Nailis *et al*., 2006)] and 60 *C. elegans* genes [including three housekeeping genes, *rps-2*, *rps-4*, *rps-23* (Tao *et al*., 2020)]. The full list of genes with functions and the sources of the 183-genes can be found in Supplementary Table S1. Analyses of differential expression was performed using nCounter®

with Elements™ XT Reagents according to manufacturer's specifications. A multiplexed probe library (nCounter® elements CodeSet) was designed with two sequence-specific probes for genes of interest. Probes were mixed with approximately 100 ng of purified total RNA and allowed to hybridize (20 h, 67 °C). Samples were loaded on an nCounter® SPRINTTM Cartridge and processed with an nCounter® SPRINT Profiler (NanoString Technologies, USA) to quantify the transcripts. The nCounter raw expression data file (RCC) obtained was uploaded into the nSolver Analysis Software 4.0 for review of quality control metrics. The data was grouped between the experiments and control, and their expression ratio was determined. Genes with a fold change of ≥ 1.5 or ≤ -1.5 were identified from the gene list in each of the experimental conditions, and only the significantly differentially expressed genes ($P \leq 0.05$) were considered.

3.8. Statistical analysis

Student's *t*-test (two-tailed, unequal variance) was used to analyse the significance of differences between experimental groups. Data with a *P*-value of ≤ 0.05 was considered to be significant. For NanoString nCounter® analysis, genes with a fold change of ≥ 1.5 or ≤ -1.5 and *P*-values ≤ 0.05 indicate a significant difference from control.

4. Results and Discussions

4.1. Exogenous 17,18-EpETE decreases hyphal formation *in vitro*

According to literature, EPA is known to be the predominant polyunsaturated fatty acid (PUFA) in *C. elegans* (Kulas *et al*., 2008). In our previous chapter, we showed that supplementation with EPA resulted in significant changes in FA profiles during infection of *C. elegans* (Chapter 3, Figure 3.3). We also showed that EPA supplementation resulted in a partially reduced *C. albicans* virulence by influencing the early stages of infection in *C. elegans* (Chapter 3, Figure 3.4). Moreover, EPA supplementation also inhibited hyphal formation of *C. albicans*. The EPA metabolite, 17,18-EpETE, that is synthesized through CYP450 enzymatic activity, is known to possess anti-allergic and anti-inflammatory effects in some diseases found in skin and gut (Kunisawa *et al*., 2015; Serhan, 2017; Saika *et al*., 2019). 17,18-EpETE is an abundant CYP eicosanoid in *C. elegans* and is produced by CYP-33E2, closely related to human CYP2J2 (Kosel *et al*., 2011) as well as CYP-29A3 (Kulas *et al*., 2008). These CYP450s prefer EPA over arachidonic acid (AA, 20:4n-6) as a substrate (Schwarz *et al*., 2004, 2005; Kosel *et al*., 2011; Liberman *et al*., 2020). In this study, we determined the effect of 17,18-EpETE on hyphal formation by exposing *C. albicans* yeast cells to 17,18-EpETE *in vitro*. As depicted by figure

4.2, addition of 17,18-EpETE to *C. albicans* yeast cells in either foetal bovine serum or nematode broth, caused a significant decrease in germ tube formation compared to control cells, indicating the possibility that production of this eicosanoid from EPA may explain the reduced hyphal formation in *C. elegans*.

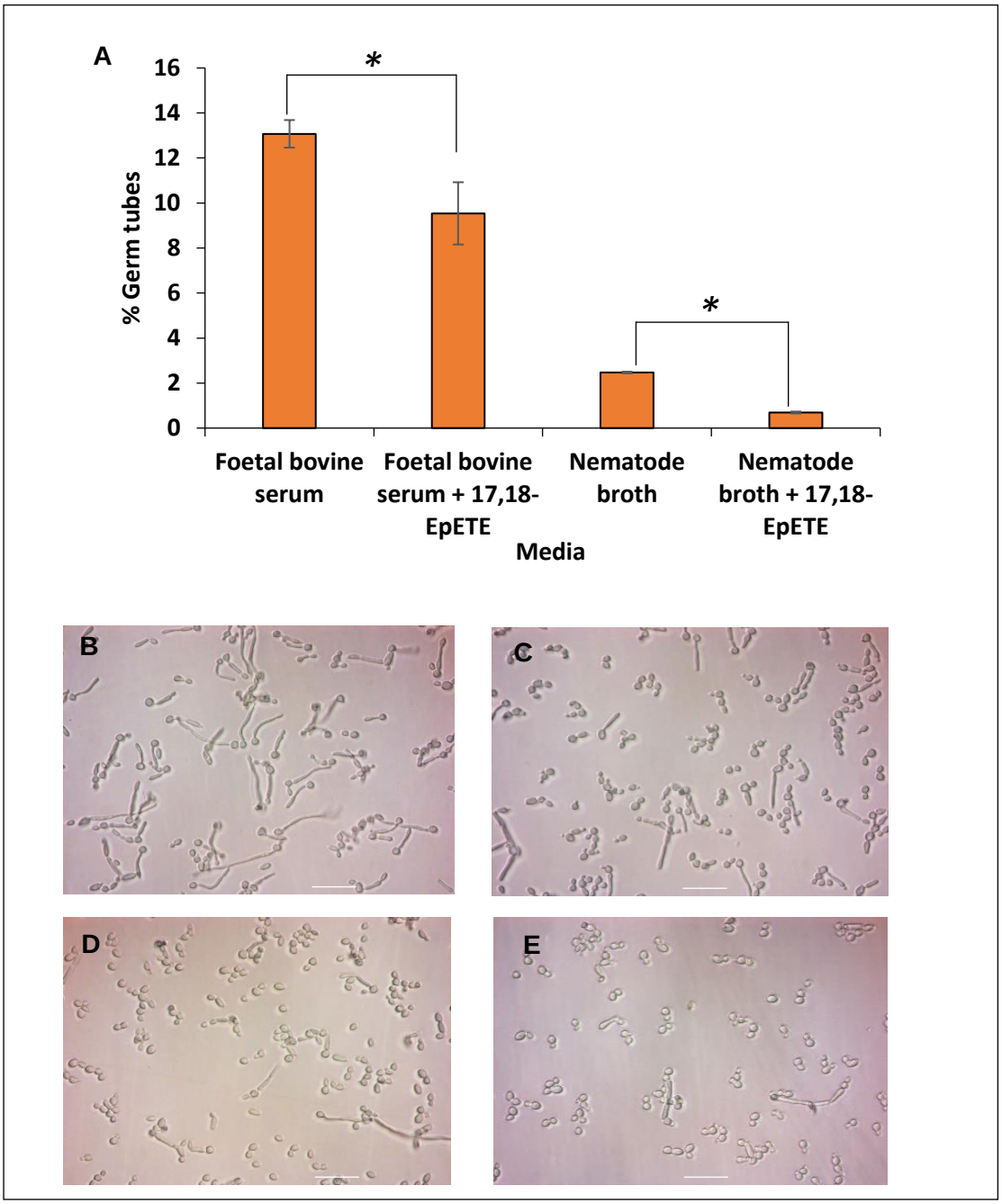


Figure 4.2. (A) Effect of 17,18-epoxy eicosatetraenoic acid (17,18-EpETE) on germ tube formation of *Candida albicans.* Values represents the mean of three independent experiments and error bars represent the standard deviations. Asterisk (*) indicate $P < 0.05$ compared to 17,18-EpETE unsupplemented media. (B) Germ tubes of *C. albicans* cultivated in foetal bovine serum. (C) Germ tubes of *C. albicans* cultivated in foetal bovine serum treated with

17,18-EpETE. (D) Germ tubes of *C. albicans* cultivated in nematode broth. (E) Germ tubes of *C. albicans* cultivated in nematode broth treated with 17,18-EpETE.

4.2. Exogenous 17,18-EpETE inhibits hyphal formation *in vivo*

To gain further insight into the role of 17,18-EpETE in *C. albicans* hyphal formation *in vivo*, we used *C. elegans* as an infection model (Tampakakis *et al*., 2008; Pukkila-Worley *et al*., 2009). In this study, nematodes infected with *C. albicans* yeast cells, were supplemented with 17,18-EpETE, while unsupplemented nematodes were used as control (Figure 4.3A). Similar to EPA supplemented nematodes, infected with *C. albicans* (Figure 4.3B), no hyphal formation was observed in 17,18-EpETE supplemented nematodes infected with *C. albicans* (Figure 4.3C). It was observed that 17,18-EpETE supplemented nematodes contained *C. albicans* yeast cells in the gut, suggesting that 17,18-EpETE supplementation inhibited the yeast to hyphal conversion, thus inhibiting the formation of invasive hyphae of *C. albicans* emerging from nematodes. This is similar to our *in vitro* results (Figure 4.2), however the complete inhibition observed *in vivo* suggest that there might be additional or enhanced inhibitory compounds or processes in the nematode.

Figure 4.3. Effect of 17,18-epoxy eicosatetraenoic acid (17,18-EpETE) on hyphal formation of *Candida albicans* infected *Caenorhabditis elegans*. (A) *C. albicans* SC5314 infected control nematodes with hyphal production. (B) Eicosapentaenoic acid (EPA) supplemented *C. albicans* SC5314 infected nematodes with no hyphal production. (C) 17,18-EpETE supplemented *C. albicans* SC5314 infected nematodes with no hyphal production. Scale bars represent 200 µm.

4.3. Exogenous CYPs inhibitors restores hyphal formation *in vivo*

Selective soluble epoxide hydrolase inhibitors, including the selective inhibitor of CYP epoxygenase, *N*-methylsulfonyl-6-(2-proparglyoxylphenyl)hexanamide (MS-PPOH), are the most commonly used compounds to study the functional effects of PUFA epoxides (Brand-Schieber *et al*., 2000; Chiamvimonvat *et al*., 2007; Morisseau *et al*., 2010; Wagner *et al*., 2011). In this part of the study, we tested the hypothesis that CYP inhibitors, 17-ODYA and PPOH (two compounds frequently used to block mammalian CYP isoforms involved in EPA metabolism), will inhibit the *C. elegans* CYP450 activity, responsible for epoxidation of EPA to 17,18-EpETE, and restore *C. albicans* hyphal formation (Figure 4.4A). In agreement with this hypothesis, our results showed that the addition of either PPOH (Figure 4.4B) or 17-ODYA (Figure 4.4C) caused *C. albicans* hyphal formation to be partially restored. Therefore, the suppression of EPA-derived metabolite (17,18-EpETE) through CYP450 inhibitors could explain the physiologically beneficial role of EPA in the virulence of *C. albicans*. This hypothesis is strengthened by the above evidence of *in vitro* and *in vivo* inhibition of *C. albicans* hyphae by 17,18-EpETE (Figure 4.2 and 4.3). By demonstrating that EPA can be metabolised by CYP450 to form 17,18-EpETE and inhibit *C. albicans* hyphal formation, while CYP450 inhibitors can restore hyphal formation, the present study provided new clues for further investigations about the physiological effects of major FAs and their metabolic derivatives on infection.

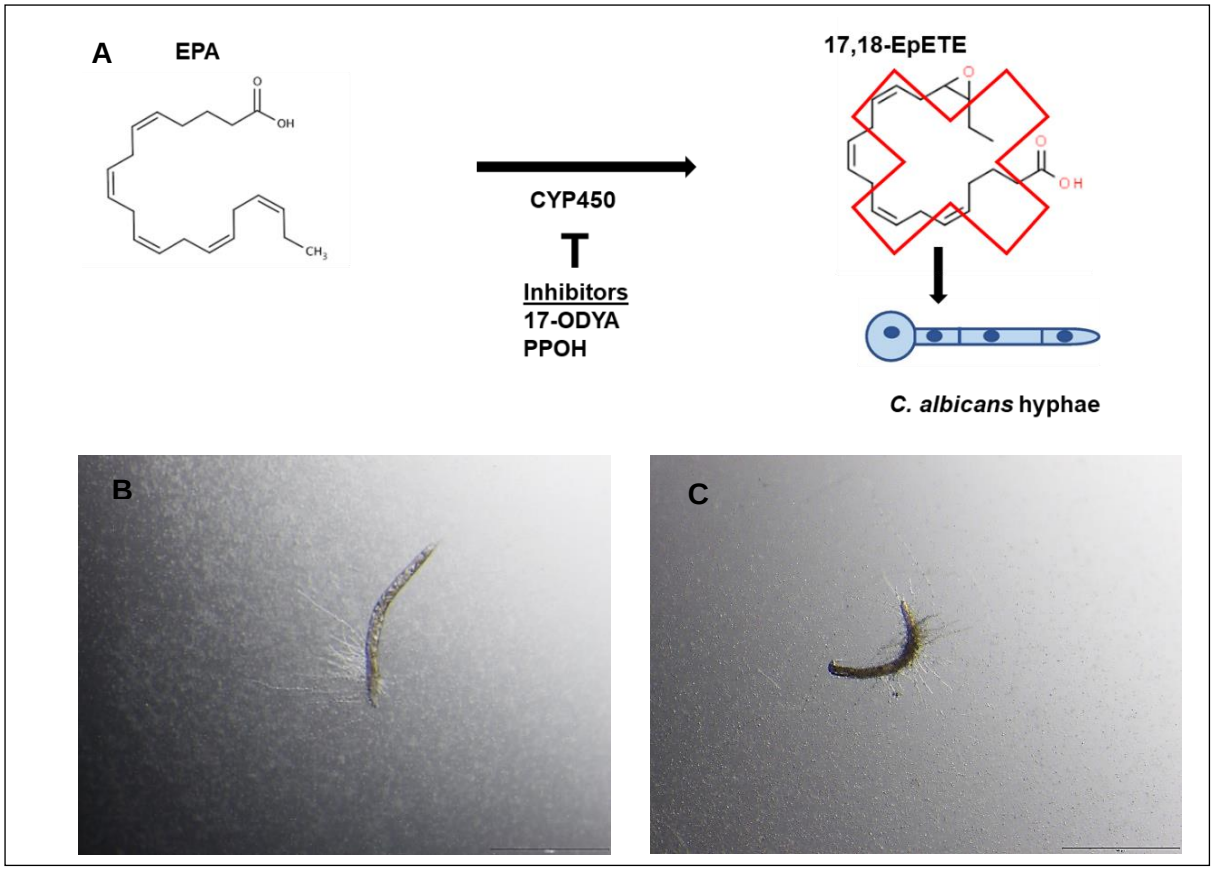


Figure 4.4. Effect of cytochrome P450 (CYP450) inhibitors on hyphal formation of *C. albicans* infected *C. elegans*. (A) 17-octadecynoic acid (17-ODYA) or 6-(2-propargyloxyphenyl)hexanoic acid (PPOH) inhibit the CYP450 to epoxidize eicosapentaenoic acid (EPA), thus EPA is not converted to 17,18-epoxy eicosatetraenoic acid (17,18-EpETE) and *C. albicans* hyphal formation restored. (B) EPA supplemented *C. albicans* infected nematodes treated with PPOH. (C) EPA supplemented *C. albicans* infected nematodes treated with 17-ODYA. Scale bars represent 200 µm.

4.4. Differential gene expression

4.4.1. The effect of EPA supplementation on gene expression in *C. elegans*

Over the decades, gene expression dynamics have provided foundational insight into almost all biological processes. Here, we analyse expression of responsive genes and transcription factor genes to infer signals and pathways that drive pathogen gene regulation during invasive *C. albicans* infection in *C. elegans* host model. Our previous work aimed at identifying the role of EPA on *C. albicans* hyphal formation, and our results suggested that EPA is likely to inhibit *C. albicans* hyphal formation (Chapter 3, Section 4.4.1). Moreover, it was also observed that

supplementation of 17,18-EpETE (major product of EPA) inhibited the formation of invasive hyphae of *C. albicans* emerging from nematodes (Chapter 4, Section 4.2). According to literature, *C. elegans* can be used to identify both virulence-related microbial genes and immune-based host genes (Kurz and Ewbank, 2003; Sifri *et al*., 2005). Thus, in this study, we first focused on the role of EPA supplementation on gene expression in *C. elegans*. Figure 4.5 depicts the overlap of up-regulated genes of uninfected EPA supplemented *C. elegans* (EPA vs control) and infected EPA supplemented *C. elegans* (*C. albicans* + EPA vs *C. albicans*). Note that no genes were significantly down-regulated under these conditions.

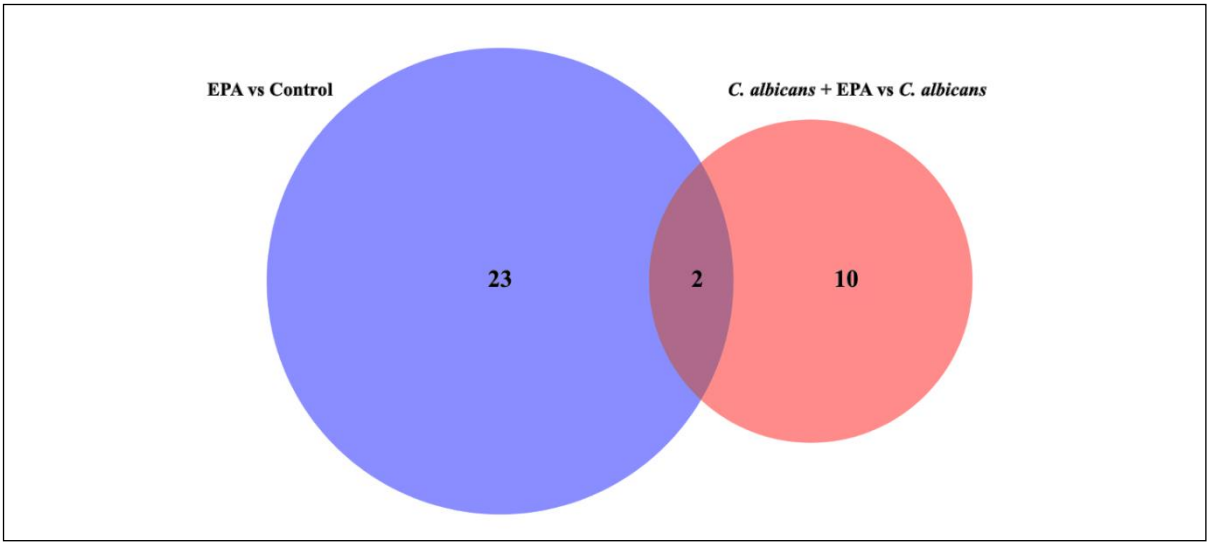


Figure 4.5. Venn diagram depicting the overlap of up-regulated genes of *Caenorhabditis elegans* genes in response to eicosapentaenoic acid (EPA) supplementation in the absence (EPA vs control) and presence (*C. albicans* + EPA vs control) of *Candida albicans* infection. Control nematodes are *E. coli* OP5O unsupplemented nematodes. See Tables 4.1 and 4.2 for gene identities.

4.4.1.1. The effect of EPA supplementation on uninfected *C. elegans*

Firstly, we determined changes in gene expression by comparing the EPA supplemented nematodes with the control nematodes (*E. coli* OP5O unsupplemented nematodes). We found that 25 genes were significantly (fold change of ≥ 1.5 and *P*-values ≤ 0.05) up-regulated (Table 4.1). Among the up-regulated genes, we observed several genes with potential roles in detoxification or antimicrobial activities (*abf-2*, *abf-3*, *cht-1*, *cyp-14A2*, *cnc-4* and *col-179*) (Table 4.1), consistent with their involvement in a protective host response (Supplementary Table S1). Other immune response genes included *cyp-37B1*, *daf-16*, *fipr-22*, *ilys-2*, *lys-5*, *lys-*

6 and *spp-12* (Table 4.1). This may indicate that supplementation with EPA primes the immune response of *C. elegans*. Several genes involved in lipid metabolism, including desaturase (*fat*) genes (*fat-2*, *fat-5* and *fat-6*), elongase (*elo*) genes (*elo-3*, *elo-7* and *elo-8*), and cytochrome P450 genes (*cyp-29A3*, *cyp-33C1* and *cyp-33E1*) were also significantly up-regulated (Table 4.1). Interestingly, none of the selected genes were down-regulated when uninfected nematodes were supplemented with EPA (Table 4.1).

Table 4.1. *Caenorhabditis elegans* genes differentially expressed by eicosapentaenoic acid supplementation.

Gene expression	Genes	Fold change	*P*-value	Lower 95% CI	Upper 95% CI
Up-regulated	*abf-2*	5.58	0.0129	1.90	16.36
	abf-3	4.50	0.0112	1.59	12.74
	cht-1	5.99	0.0051	2.17	16.49
	cnc-4	3.11	0.0445	1.07	9.02
	col-179	2.63	0.0407	0.96	7.23
	cyp-14A2	6.13	0.0099	1.77	21.21
	cyp-29A3	6.03	0.0073	2.33	15.57
	cyp-33C1	5.05	0.0245	1.19	21.39
	cyp-33E1	4.91	0.0123	1.53	15.84
	cyp-37B1	4.96	0.0150	1.44	17.05
	daf-16	5.64	0.0369	0.76	41.71
	elo-3	5.14	0.0195	1.18	22.32
	elo-7	5.16	0.0076	2.08	12.78
	elo-8	4.80	0.0092	1.72	13.44
	emb-8	4.36	0.0172	1.34	14.24
	fat-2	1.66	0.0094	1.20	2.29
	fat-5	3.36	0.0073	1.8	6.26
	fat-6	2.40	0.0161	1.24	4.62
	fipr-22	2.92	0.0242	1.41	6.09
	ilys-2	4.68	0.0192	1.34	16.38
	lys-5	4.24	0.0193	1.40	12.88
	lys-6	4.51	0.0337	1.12	18.22
	mboa-7	5.95	0.0025	2.72	13.01

	nhr-49	5.11	0.0081	1.82	14.39
	spp-12	6.29	0.0074	2.26	17.55
Down-regulated	No genes differentially regulated				

The table represents genes with a fold change of ≥ 1.5. *P*-values ≤ 0.05 indicate a significant difference from control, with lower and upper percentage confidence intervals (CI).

As stated in literature, *fat-2* encodes the Δ12 desaturase enzyme, which facilitates the biosynthesis of linoleic acid (18:2n-6) from its substrate, oleic acid (18:1n-9) (Zhou *et al*., 2011; Wang *et al*., 2013). Therefore, this observed up-regulation of *fat-2* corresponds to the observed increase in the relative percentage of 18:2n-6 ($P < 0.005$) in EPA supplemented nematodes with *E. coli* compared to unsupplemented nematodes with *E. coli* as seen in our previous chapter (Figure 4.6A). In addition, it may explain the observed increase in α-linolenic acid (18:3n-3) (Figure 4.6A), as the FA metabolism is rerouted along this branch of the pathway (Figure 4.6B). Notably, n-3 and n-6 PUFAs compete for desaturation and chain elongation at all steps of this pathway (Konkel and Schunck, 2011). The *fat-5* desaturase particularly acts on palmitic acid (16:0) producing palmitoleic acid (16:1n-7), which can further be elongated to *cis*-vaccenic acid (18:1n-7) (Watts and Browse, 2000). It is also known that *fat-6* and *fat-7* desaturases act on stearic acid (18:0) synthesizing 18:1n-9 (Watts and Browse, 2000) and up-regulation of *fat-6* correlates to the observed decrease in the relative percentage of 18:0 and increase in 18:1n-9, although these changes were not statistically significant (Figure 4.6A). The up-regulation of *fat-5* and *fat-6* in the presence of PUFAs was also seen in a recent study by Wang and co-workers (2022). Furthermore, supplementation with EPA resulted in the up-regulation of *cyp-29A3* (Table 4.1), which encodes one of the two major CYPs that oxidizes EPA to eicosanoids, including 17,18-EpETE (Kulas *et al*., 2008). These eicosanoids are key stress signalling molecules (Liberman *et al*., 2020) and as indicated above (Section 4.2), may be responsible for the inhibition of *C. albicans* hyphal formation. Moreover, amongst these up-regulated genes observed in EPA supplemented nematodes, was *nhr-49* (Table 4.1), which is involved in determination of adult lifespan, positive regulation of transcription from RNA polymerase II promoter in response to stress, regulation of FA metabolic process and immuno-metabolic response to bacterial infection (Van Gilst *et al*., 2005). Goh and co-workers (2018) suggested that, in addition to its role in regulating lipid metabolism, *nhr-49* participates in a cytoprotective acute stress response program that functions parallel to and independently of SKN-1/Nrf2 and HLH-30/TFEB signalling (Lapierre

et al., 2013; Blackwell *et al*., 2015). This raises the possibility that the pro-longevity function of *nhr-49*, may involve modulating both lipid metabolism and stress defences (Ratnappan *et al*., 2014). Interestingly, low levels of oxidative stress have been reported to increase the lifespan of *C. elegans* (Wei and Kenyon, 2016), while high levels decrease lifespan (Schaar *et al*., 2015).

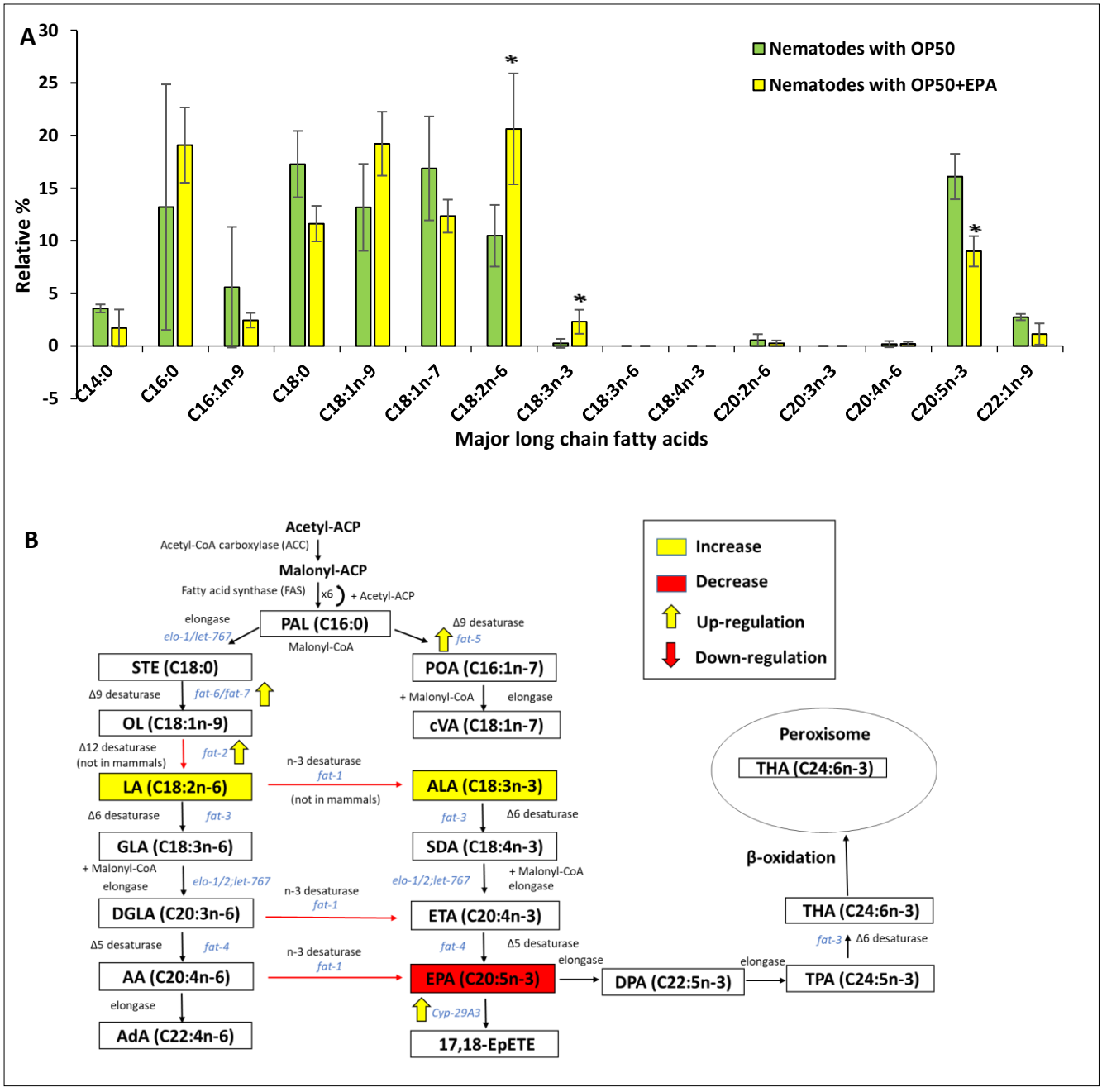


Figure 4.6. (A) Effect of eicosapentaenoic acid (EPA) supplementation on fatty acid composition of major long chain fatty acids of *Caenorhabditis elegans*. Values represent the mean of three independent experiments and error bars represents the standard deviations. Asterisk (*) indicate $P < 0.05$ compared to unsupplemented nematodes. (B) Effect of EPA on

C. elegans polyunsaturated fatty acids biosynthesis pathway. Yellow squares indicate significant increase, while red squares indicate significant decrease in relative percentage of fatty acids compared to unsupplemented nematodes with *Escherichia coli* OP50. Yellow arrows indicate up-regulation, while red arrows indicate down-regulation in genes compared to unsupplemented nematodes with *E. coli* OP50.

4.4.1.2. The effect of EPA supplementation on *C. albicans* infected nematodes

Comparing EPA supplemented nematodes infected with *C. albicans* to unsupplemented *C. albicans* infected nematodes, we discovered that *fat-3*, *fat-4* and *fat-6*, involved in the synthesis of FAs, were significantly up-regulated ($P \leq 0.05$) in response to EPA supplementation (Table 4.2). According to Watts and Browse (2002), *fat-3* encodes a delta-6 FA desaturase that is required for the biosynthesis of 18:3n-6 and 18:4n-3. While, *fat-4* encodes a delta-5 FA desaturase, which functions to modify 20-carbon PUFAs to produce AA and EPA. Moreover, fat-4(wa14) mutants are unable to generate AA or EPA (Watts and Browse, 1999; Kahn-Kirby *et al*., 2004). Interestingly, in our previous chapter, we detected low levels of the relative percentage of AA in unsupplemented *C. albicans* nematodes, however when these nematodes where supplemented with EPA, there was an insignificant increase in AA relative percentage (Figure 4.7A). This result might explain the observed up-regulation of *fat-4* (Table 4.2 and Figure 4.7B). Moreover, *elo-6* and *elo-9*, encoding putative very long chain FA elongases, were also significantly up-regulated ($P \leq 0.05$) in EPA nematodes infected with *C. albicans* (Table 4.2). *elo-6* is required for the formation of iso-heptadecanoic acid, important for nematode development and growth (Kniazeva *et al*., 2004), while *elo-9* is involved in stress responses and lifespan increase (Fajardo *et al*., 2019). We also observed a significant up-regulation ($P \leq 0.05$) of CYP450 genes, *cyp-29A2* and *cyp-37A1* (Table 4.2), which are expressed in the intestine and play a role in lipid storage and life span (Zhang *et al.,* 2013). Supplementation of EPA also influenced the expression of *cyp-14A2* (Table 4.2), involved in stress response and detoxification (Thomas, 2007). Therefore, we can speculate that supplementation with PUFAs can cause oxidative stress, which influenced expression of genes involved stress response and life span described above.

Furthermore, it is known that the host response to *C. albicans* involves induction of specific defences and common immune genes (Pukkila-Worley *et al*., 2011). Since EPA supplementation prior to infection with *C. albicans*, significantly extended the time needed to kill 50% of the nematodes compared to unsupplemented infected nematodes, as seen in chapter 3, section 4.4.1, the effect of EPA supplementation on the host immune response to

infection was also investigated. Among the effector genes significantly up-regulated ($P \leq 0.05$) in EPA nematodes infected with *C. albicans* (Table 4.2), were those that had previously been demonstrated to be involved in *C. elegans* immunity, such as C-type lectin genes, *clec-60* and *clec-67*, suggesting a vital role for these antimicrobial peptides in the defence of *C. elegans* against *C. albicans* infection. *clec-67* was previously shown to be part of the *P. aeruginosa* transcriptional response (Troemel *et al*., 2006), however strongly down-regulated after *C. albicans* infection (Pukkila-Worley *et al*., 2011). While *clec-60* is a gene class involved in nematode defence response to Gram-positive bacteria (Troemel *et al*., 2006; Bolz *et al*., 2010; Irazoqui *et al*., 2010). Therefore, we conclude that the nematodes up-regulate a group of antibacterial defence genes in response to some aspect of *C. albicans* virulence. Although the differential expression of these genes in bacterial infections offers clues to their potential functions, the elucidation of their exact function in *C. albicans* will require further study. Furthermore, *C. albicans* infection of the EPA supplemented nematodes caused the up-regulation of caenopore genes, *spp-2* (involved in defence response to Gram-positive bacteria) and *spp-14* (involved in immune response) (Table 4.2). Given all this, our dataset suggest that the nematodes are able to specifically recognize *C. albicans* infection and mount a targeted response toward this fungus, thus involving antibacterial genes and a limited number of common core effectors. Therefore, this provides another line of evidence that yeast infection of the nematodes stimulates host immune defences.

We also looked for overlap between the 36 genes up-regulated in different conditions. As can be seen in figure 4.5, two *C*. *elegans* genes, *fat-6* and *cyp-14A2*, are up-regulated in both datasets. When looking at the levels of up-regulation of *cyp-14A2*, we see that the presence of *C. albicans* in EPA supplemented nematodes, caused a decrease in up-regulation (Table 4.2), with the fold change in EPA vs control (6.13) (Table 4.1) decreasing to 4.15 (Table 4.2). The addition of *C. albicans* to the EPA supplemented nematodes did not greatly influence the up-regulation of *fat-6* [EPA vs control fold change = 2.4 (Table 4.1); *C. albicans* + EPA vs *C. albicans* fold change = 2.22 (Table 4.2)], implying that this gene is more responsive to EPA than to *C. albicans* infection.

Table 4.2. *Caenorhabditis elegans* genes differentially expressed by eicosapentaenoic acid supplementation during *Candida albicans* infection.

Gene expression	Genes	Fold change	*P*-value	Lower 95% CI	Upper 95% CI
Up-regulated	*clec-60*	2.35	0.0425	1.02	5.41
	clec-67	4.84	0.0247	1.63	14.37
	cyp-14A2	4.15	0.0305	1.39	12.40
	cyp-29A2	5.15	0.0020	2.52	10.53
	cyp-37A1	5.05	0.0244	1.67	15.33
	elo-6	1.98	0.0306	1.03	3.81
	elo-9	6.49	0.0022	2.75	15.30
	fat-3	1.87	0.0413	0.92	3.80
	fat-4	2.40	0.0039	1.61	3.59
	fat-6	2.22	0.0339	1.11	4.46
	spp-2	3.44	0.0038	1.85	6.40
	spp-14	1.76	0.0018	1.40	2.22
Down-regulated	*elo-5*	-1.82	0.0372	0.33	-1.09

The table represents genes with a fold change of ≥ 1.5. *P*-values ≤ 0.05 indicate a significant difference from unsupplemented *C. albicans* infected nematodes with lower and upper percentage confidence intervals (CI).

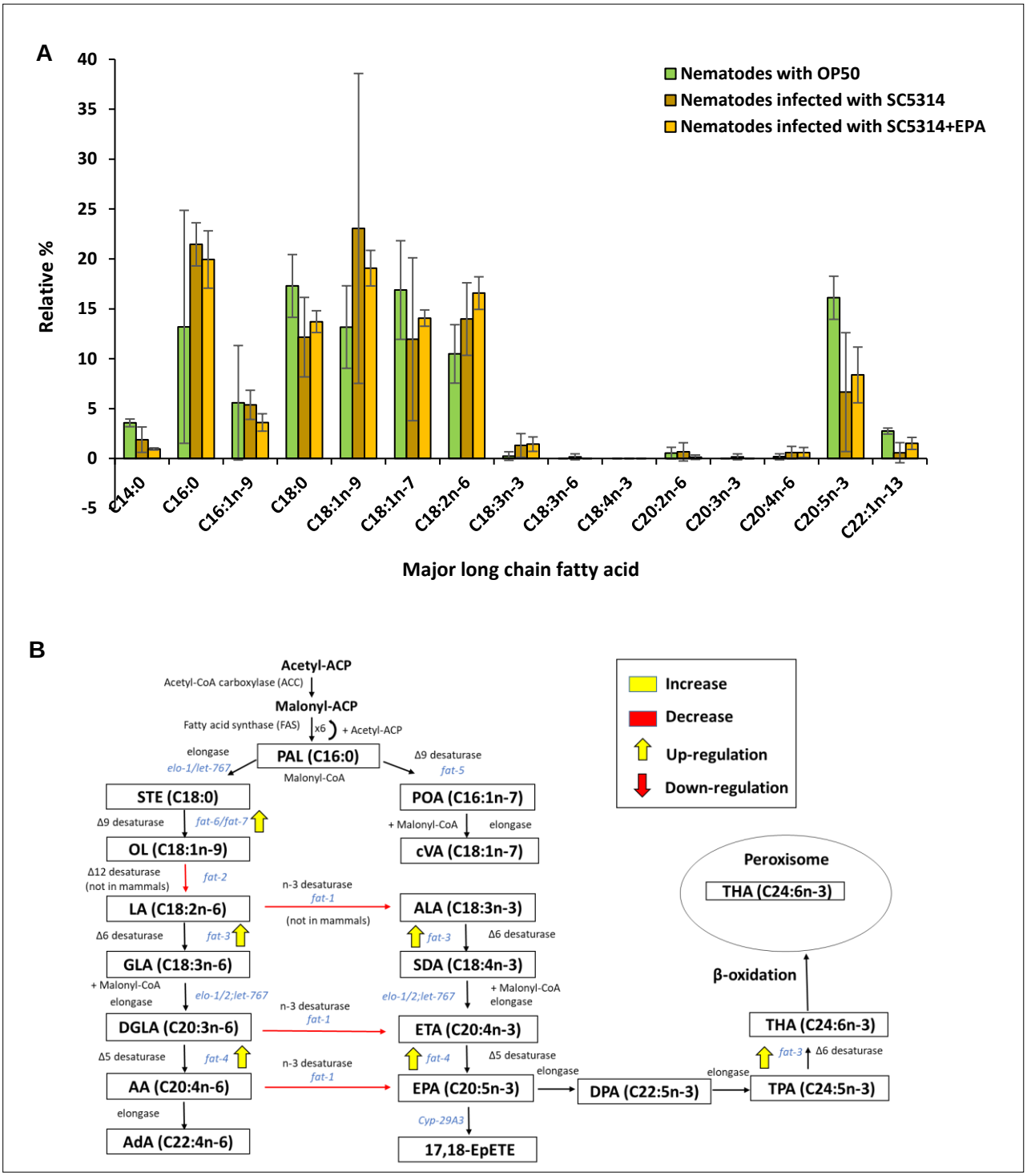


Figure 4.7. (A) Effect eicosapentaenoic acid (EPA) supplementation on fatty acid composition of major long chain fatty acids of *Candida albicans* infected *Caenorhabditis elegans*. Values represent the mean of three independent experiments and error bars represent the standard deviations. (B) Effect of EPA on *C. albicans* infected *C. elegans* polyunsaturated fatty acids biosynthesis pathway. Yellow squares indicate significant increase, while red squares indicate significant decrease in relative percentage of fatty acids compared to unsupplemented *C. albicans* infected nematodes. Yellow arrows indicate up-regulation, while red arrows indicate down-regulation in genes compared to unsupplemented *C. albicans* infected nematodes.

4.4.2. The effect of EPA on *C. albicans* gene expression

Morphogenesis contributes to the virulence of *C. albicans*, thus yeast to hyphal conversion is an important virulence property of *C. albicans* (Gow *et al*., 2002). The formation of hyphae aids the subsequent invasive growth of *C*. *albicans* to penetrate host tissues and lead to the establishment of systemic infection (Saville *et al*., 2003). Importantly, hyphal morphogenesis in *C. albicans* is tightly controlled by dozens of transcription factors that contribute to the activation or repression of the hyphal transcriptional program (Villa *et al*., 2020). In chapter 3, we showed that supplementation with EPA resulted in a partially reduced *C. albicans* virulence by influencing the early stages of infection in *C. elegans*. Moreover, EPA supplementation also inhibited hyphal formation of *C. albicans*. Given this, in the current chapter, we investigated the change in gene expression changes elicited by EPA on *C. albicans*, with a focus on genes involved in morphogenesis. The genes that are differentially expressed in response to EPA is given in Table 4.3 (up-regulated genes) and Table 4.4 (down-regulated genes). Transcription-profiling analysis of *C. albicans* during *C. elegans* infection in the presence of EPA, caused a significant response involving 112 genes (74 up-regulated and 38 down-regulated).

Inhibition of *C. albicans* hyphal formation by the FAs isolated from whey cream, mainly 12:0, myristoleic acid, 16:1n-7, 18:2n-6 and AA, was previously shown (Clement *et al*., 2007). Similarly, Kuloyo and co-workers (2020) showed that AA inhibits *C. albicans* hyphal formation. In chapter 2, survival studies revealed that every *C. albicans* infected nematode that was killed during the first 2 days had visible hyphae piercing the cuticle (Chapter 2, Figure 2.1B-C). However, in chapter 3, we observed that supplementation of EPA inhibits *C. albicans* filamentation (Chapter 3, Figure 3.4D), thus, in this current study, we analysed changes of gene expression levels in *C. albicans* during infection of nematodes supplemented with EPA relative to unsupplemented nematodes. We discovered that 74 *C. albicans* genes were significantly up-regulated (Table 4.3). Amongst these genes, 64 genes are involved in hyphal formation (Supplementary Table S1). Similar results were seen by Kuloyo and co-workers (2020), where the addition of the PUFA, AA, caused an up-regulation of genes involved in biofilm and hyphal formation even though exposure to this PUFA inhibited morphogenesis. This was seen as a means to compensate for the hyphal inhibitory effect of AA. Genes involved in hyphal or biofilm formation that were up-regulated by to EPA *in vivo* and AA *in vitro* are *CRZ1*, *FCR1*, *RTA3*, *SNQ2* and *TEC1*. In addition, other genes found by Kuloyo and co-workers (2020) to be up-regulated in the presence of AA *in vitro*, were also up-regulated by the presence of EPA *in vivo*. These genes were *ETH1*, *FLU1*, *IPT1* and *SUT1* (Table 4.3). This correlation between the two datasets may indicate that expression of at least some of

these genes may be regulated by the addition of PUFAs both *in vitro* and *in vivo*. Interestingly, some genes showed an inverse regulation in comparison with the data of Kuloyo and co-workers (2020). These genes were *ALS1*, *TYE7* and *YWP*, which were up-regulated *in vitro* by AA, but down-regulated by EPA *in vivo* (Table 4.4). Moreover, *ECM17* and *SUL2* were down-regulated by AA *in vitro*, but up-regulated by EPA *in vivo* (Table 4.3). The specific roles of these genes during infection in the presence of EPA needs further study.

Table 4.3. *Candida albicans* genes up-regulated by eicosapentaenoic acid *in vivo.*

Conditions	**Genes**	**Fold change**	***P*-value**	**Lower 95% CI**	**Upper 95% CI**
C. albicans* + EPA vs *C. albicans	*ACE2*	3.83	0.0003	2.93	5.00
	ADR1	2.96	0.0087	1.47	5.99
	AFT2	3.26	0.0056	1.63	6.53
	CAS5	2.66	0.0047	1.60	4.41
	CPH1	3.60	0.00002	3.50	3.70
	CRZ1	3.23	0.0025	1.85	5.64
	CSR1	4.25	0.0013	3.39	5.32
	CTA4	2.16	0.0023	1.60	2.92
	CWT1	2.42	0.0104	1.48	3.95
	DAL8	4.62	0.0002	3.17	6.73
	EHT1	2.36	0.0189	1.21	4.61
	ECM17	2.59	0.0255	0.97	6.97
	ERG11	2.14	0.0288	1.20	3.80
	FAH2	2.83	0.0014	1.85	4.32
	FCR1	1.62	0.0061	1.23	2.14
	FGR13	2.77	0.0050	2.01	3.81
	FGR17	3.18	0.0001	2.64	3.83
	FGR27	2.49	0.0229	1.13	5.53
	FKH2	2.93	0.0039	1.73	4.95
	FLU1	2.57	0.0018	2.16	3.06
	HAP5	3.95	0.0001	3.06	5.10
	HRD3	1.99	0.0030	1.53	2.59
	CTA8	1.84	0.0186	1.20	2.82
	HWP1	3.63	0.00009	2.89	4.56

	IPT1	2.79	0.0064	1.95	3.99
	LSC2	2.02	0.0005	1.70	2.39
	MDR1	2.70	0.0295	1.27	5.71
	MET4	2.84	0.0116	1.55	5.18
	NGS1	3.40	0.0335	1.17	9.90
	NOT3	3.35	0.0006	2.95	3.82
	OPI1	2.19	0.0205	1.15	4.17
	PPR1	3.12	0.0017	2.02	4.82
	PST1	3.94	0.0005	2.61	5.95
	RBF1	3.15	0.0054	1.74	5.70
	RCA1	1.93	0.0064	1.35	2.77
	RFX2	3.12	0.0044	2.24	4.33
	RGT1	2.36	0.0203	1.07	5.19
	RLM1	3.40	0.0010	2.17	5.32
	RON1	4.63	0.0096	2.41	8.90
	RTA3	3.77	0.0007	2.58	5.53
	RTG3	2.39	0.0128	1.26	4.54
	SAP6	4.15	0.0184	1.78	9.65
	SET3	2.66	0.0027	1.83	3.86
	SFL2	1.93	0.0265	1.08	3.47
	SIN3	2.88	0.0044	1.73	4.80
	SKN7	3.16	0.0159	1.25	7.96
	SKO1	2.25	0.0469	1.02	4.97
	SNF4	1.58	0.0385	1.04	2.41
	SNF5	2.92	0.0197	1.22	6.96
	SNF6	3.29	0.0065	1.62	6.67
	SNQ2	3.12	0.00009	2.53	3.85
	SPT3	2.79	0.0045	1.80	4.33
	SPT6	2.79	0.0045	1.80	4.33
	SPT20	3.16	0.0051	1.70	5.87
	STD1	2.15	0.0182	1.32	3.49
	SUL2	2.98	0.0156	1.65	5.41
	SUT1	2.53	0.0132	1.27	5.03
	SWI1	3.14	0.0092	1.67	5.91

	SWI4	2.83	0.0154	1.28	6.26
	TAC1	2.60	0.0350	1.01	6.74
	TEC1	2.12	0.0184	1.14	3.97
	TFG1	1.86	0.0191	1.11	3.14
	TUP1	1.89	0.0114	1.36	2.61
	UME6	3.67	0.0137	1.89	7.12
	WOR1	2.70	0.0403	1.11	6.53
	YOR1	1.80	0.0359	1.07	3.04
	ZCF3	3.09	0.0076	1.69	5.65
	ZCF7	3.18	0.0060	2.15	4.70
	ZCF11	3.40	0.0018	2.16	5.34
	ZCF14	3.05	0.0327	1.09	8.54
	ZCF17	3.08	0.0006	2.15	4.40
	ZCF18	3.02	0.0071	1.74	5.25
	ZCF29	2.86	0.0020	1.79	4.59
	ZCF32	3.70	0.0005	2.86	4.79

The table represents genes with a fold change of ≥ 1.5. *P*-values ≤ 0.05 indicate a significant difference from unsupplemented *C. albicans* infected nematodes, with lower and upper percentage confidence intervals (CI).

Table 4.4. *Candida albicans* genes down-regulated by eicosapentaenoic acid *in vivo.*

Conditions	**Genes**	**Fold change**	***P*-value**	**Lower 95% CI**	**Upper 95% CI**
C. albicans* + EPA vs *C. albicans	*ALS1*	-2.94	0.0010	0.23	-1.98
	CPH2	-1.92	0.0026	0.40	-1.48
	CUP9	-3.01	0.0005	0.24	-2.15
	RIM101	-2.64	0.0015	0.26	-1.80
	STP2	-1.69	0.0253	0.38	-1.08
	TDH3	-1.95	0.00004	0.46	-1.76
	TYE7	-2.87	0.0017	0.22	-1.85
	YWP1	-1.97	0.0078	0.37	-1.42

The table represents genes with a fold change of ≥ 1.5. *P*-values ≤ 0.05 indicate a significant difference from unsupplemented *C. albicans* infected nematodes, with lower and upper percentage confidence intervals (CI).

5. Conclusions

From our previous chapter, we revealed that supplementation with EPA resulted in a partially reduced *C. albicans* virulence in *C. elegans* and inhibition of hyphal production of *C. albicans*. On the basis of these findings, in this study, we hypothesized that CYP450 epoxygenase metabolize EPA to 17,18-EpETE which inhibits *C. albicans* hyphal formation. We used both *in vitro* and *in vivo C. elegans* model to confirm the anti-hyphal efficacies of EPA metabolite, 17,18-EpETE and found that 17,18-EpETE resulted in the inhibition of *C. albicans* hyphal formation both *in vitro* and *in vivo*. In addition, treatments with inhibitors of mammalian EPA-metabolising CYP enzymes, 17-ODYA and PPOH, significantly restored *C. albicans* hyphal formation in infected *C. elegans*. These results demonstrated that EPA and 17,18-EpETE are efficient in inhibiting hyphal formation of *C. albicans*. This would provide the opportunity to use *C. elegans* as a model organism to facilitate the identification of the primary targets of FAs and their metabolites, and the elucidation of signalling pathways mediating their physiological functions. Furthermore, the role of EPA on the physiology of *C. elegans* as well as *C. albicans in vivo* was investigated using gene expression analyses. Among the up-regulated genes, we observed several genes with potential roles in detoxification or antimicrobial activities, consistent with their involvement in a protective host response. This may indicate that supplementation with EPA may prime the immune response of *C. elegans*. Moreover, several genes involved in lipid metabolism, including *fat* genes, *elo* genes and cytochrome P450 genes were also significantly up-regulated. We can speculate that these results indicate a response, where decrease in long chain FA levels could induce increased expression of corresponding biosynthetic genes. Interestingly, there was an up-regulation of *cyp-29A3*, which encodes an enzyme that oxidizes EPA to eicosanoids (Liberman *et al*., 2020), such as 17,18-EpETE. Furthermore, gene expression analysis of *C. albicans* genes regulated by EPA supplementation revealed that majority of the genes involved in hyphal growth, were significantly up-regulated, which may be an attempt by the yeast to compensate for the inhibitory effect of EPA or 17,18-EpETE. Given all this, it is clear that EPA significantly induces alterations in lipid metabolism and immune response of *C. elegans* as well as hyphal formation in *C. albicans*.

6. References

Bergsson, G., Arnfinnsson, J., Steingrimsson, O. & Thormar, H. (2001). *In vitro* killing of *Candida albicans* by fatty acids and monoglycerides. *Antimicrob Agents Chemother* **45**, 3209-3212.

Berman, J. & Sudbery, P. E. (2002). *Candida albicans*: a molecular revolution built on lessons from budding yeast. *Nat Rev Genet* **3**, 918–930.

Blackwell, T. K., Steinbaugh, M. J., Hourihan, J. M., Ewald, C. Y. & Isik, M. (2015). SKN-1/Nrf, stress responses, and aging in *Caenorhabditis elegans. Free Radic Biol Med* **88**, 290–301.

Bolz, D. D., Tenor, J. L. & Aballay, A. (2010). A conserved PMK-1/p38 MAPK is required in *Caenorhabditis elegans* tissue-specific immune response to *Yersinia pestis* infection. *J Biol Chem* **285**, 10832–10840.

Brand-Schieber, E., Falck, J. F. & Schwartzman, M. (2000). Selective inhibition of arachidonic acid epoxidation *in vivo*. *J Physiol Pharmacol* **51** (4), 655-72.

Braun, B. R. & Johnson, A. D. (1997). Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science* **277**, 105–109.

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M. & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *C. elegans* pathogenicity assay. *PLoS Pathog* **3**, e18.

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

Cao, F., Lane, S., Raniga, P. P., Lu, Y., Zhou, Z. *et al*. (2006). The Flo8 transcription factor is essential for hyphal development and virulence in *Candida albicans*. *Mol Biol Cell* **17**, 295–307.

Chiamvimonvat, N., Ho, C. M., Tsai, H. J. & Hammock, B. D. (2007). The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J Cardiovasc Pharmacol* **50** (3), 225-237.

Clement, M., Tremblay, J., Lange, M., Thibodeau, J. & Belhumeur, P, (2007). Whey-derived free fatty acids suppress the germination of *Candida albicans in vitro*. *FEMS Yeast Res* **7**, 276–285.

Deline, M. L., Vrablik, T. L. & Watts, J. L. (2013). Dietary supplementation of polyunsaturated fatty acids in *Caenorhabditis elegans*. *J Vis Exp* **81**, e50879.

Desbois, A. P. & Smith, V. J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Appl Microbiol Biotechnol* **85,** 1629–1642.

Fajardo, C., Costa, G., Nande, M., Martin, C., Martin, M. & Sanchez-Fortun, S. (2019). Heavy metals immobilization capability of two iron-based nanoparticles (nZVI and Fe3O4): soil and freshwater bioassays to assess ecotoxicological impact. *Sci Total Environ* **656**, 421-432.

Ford, C. B., Funt, J. M., Abbey, D., Issi, L., Guiducci, C., Martinez, D. A., Delorey, T., Li, B. Y., White, T. C., Cuomo, C., Rao, R. P., Berman, J., Thompson, D. A. & Regev, A. (2015). The evolution of drug resistance in clinical isolates of *Candida albicans*. *eLife* **4**.

Gantner, B. N., Simmons, R. M. & Underhill, D. M. (2005). Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J* **24**, 1277–1286.

Geiss, G. K., Bumgarner, R. E., Birditt, B., Dahl, T., Dowidar, N. *et al*. (2008). Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotechnol* **26**, 317–325.

Goh, G., Winter, J. J., Bhanshali, F., Doering, K., Lai, R., Lee, K., Veal, E. A. & Taubert, S. (2018). NHR-49/HNF4 integrates regulation of fatty acid metabolism with a protective transcriptional response to oxidative stress and fasting. *Aging cell* **17** (3), e12743.

Gow, N. A., Brown, A. J. & Odds, F. C. (2002). Fungal morphogenesis and host invasion. *Curr Opin Microbiol* **5**, 366–371.

Irazoqui, J. E., Troemel, E. R., Feinbaum, R. L., Luhachack, L. G., Cezairliyan, B. O. & Ausubel, F. M. (2010). Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. *PLoS Pathog* **6** (7), e1000982.

Jenkins, W. R. (1964). A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis Rep* **48**, 692.

Kahn-Kirby, A. H., Dantzker, J. L. M., Apicella, A. J., Schafer, W. R., Browse, J. *et al*. (2004) Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling *in vivo. Cell* **119**, 889–900.

Kniazeva, M., Crawford, Q. T., Seiber, M., Wang, C. & Han, M. (2004). Monomethyl branched-chain fatty acids play an essential role in *Caenorhabditis elegans* development. *PLoS Biol* **2**, E257

Konkel, A. & Schunck, W. H. (2011). Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim Biophys Acta* **1814** (1), 210-222.

Kosel, M., Wild, W., Bell, A., Rothe, M., Lindschau, C. *et al*. (2011). Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of *Caenorhabditis elegans*. *Biochem J* **435**, 689–700.

Kulas, J., Schmidt, C., Rothe, M., Schunck, W. H. & Menzel, R. (2008). Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*. *Arch Biochem Biophys* **472**, 65–75.

Kuloyo, O., Fourie, R., Cason, E., Albertyn, J. & Pohl, C. H. (2020). Transcriptome analyses of *Candida albicans* biofilms, exposed to arachidonic acid and fluconazole, indicates potential drug targets. *G3* (*Bethesda)* **10** (9), 3099–3108.

Kumamoto, C. A. & Vinces, M. D. (2005). Contributions of hyphae and hypha-coregulated genes to *Candida albicans* virulence. *Cell Microbiol* **7**, 1546–1554.

Kumar, P., Lee, J. H., Beyenal, H. & Lee, J. (2020). Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol* **28**, 753–768.

Kunisawa, J., Arita, M., Hayasaka, T., Harada, T., Iwamoto, R., Nagasawa, R. *et al*. (2015). Dietary omega-3 fatty acid exerts anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut. *Sci Rep* **5**, 9750.

Kurz, C. L. & Ewbank, J. J. (2003). *Caenorhabditis elegans*: an emerging genetic model for the study of innate immunity. *Nat Rev Genet* **4**, 380–390.

Lapierre, L. R., De Magalhaes Filho, C. D., McQuary, P. R., Chu, C. -C., Visvikis, O., Chang, J. T. & Hansen, M. (2013). The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. *Nat Commun* **4**, 2267.

Liberman, N., O'Brown, Z. K., Earl, A. S., Boulias, K., Gerashchenko, M. V., Wang, S. Y., Fritsche, C., Fady, P. E., Dong, A., Gladyshev, V. N. & Greer, E. L. (2020). N6-adenosine methylation of ribosomal RNA affects lipid oxidation and stress resistance. *Sci Adv* **6** (17), eaaz4370.

Lo, H. J., Kohler, J. R., Di Domenico, B., Loebenberg, D., Cacciapuoti, A. *et al*. (1997). Nonfilamentous *C. albicans* mutants are avirulent. *Cell* **90**, 939–949.

Morisseau, C., Inceoglu, B., Schmelzer, K., Tsai, H. J., Jinks, S. L. Hegedus, C. M. & Hammock, B. D. (2010). Naturally occurring monoepoxides of eicosapentaenoic acid and docosahexaenoic acid are bioactive antihyperalgesic lipids [S]. *J Lipid Res* **51** (12), 3481-3490.

Moyes, D. L., Runglall, M., Murciano, C., Shen, C., Nayar, D. *et al*. (2010). A biphasic innate immune MAPK response discriminates between the yeast and hyphal forms of *Candida albicans* in epithelial cells. *Cell Host Microbe* **8**, 225–235.

Murzyn, A., Krasowska, A., Stefanowicz, P., Dziadkowiec, D. & Lukaszewicz, M. (2010). Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. *PLoS One* **5**, e12050.

Muthamil, S., Balasubramaniam, B., Balamurugan, K. & Pandian, S. K. (2018). Synergistic effect of quinic acid derived from syzygium cumini and undecanoic acid against *Candida* spp. biofilm and virulence. *Front Microbiol* **9**, 2835.

Nailis, H., Coenye, T., Van Nieuwerburgh, F., Deforce, D. & Nelis, H. J. (2006). Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. *BMC Mol Biol* **7**, 25.

Nobile, C. J., Solis, N., Myers, C. L., Fay, A. J., Deneault, J. S., Nantel, A., Mitchell, A. P. & Filler, S. G. (2008). *Candida albicans* transcription factor Rim101 mediates pathogenic interactions through cell wall functions. *Cell Immunol* **1**, 2180–2196.

Noverr, M. C. & Huffnagle, G. B. (2004). Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. *Infect Immun* **72**, 6206–6210.

Prasath, K. G., Sethupathy, S. & Pandian, S. K. (2019). Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. *J Proteomics* **208**, 103503.

Pukkila-Worley, R., Ausubel, F. M. & Mylonakis, E. (2011). *Candida albicans* infection of *Caenorhabditis elegans* induces antifungal immune defences. *PLoS Pathog* **7** (6), e1002074.

Pukkila-Worley, R., Peleg, A. Y., Tampakakis, E. & Mylonakis, E. (2009). *Candida albicans* hyphal formation and virulence assessed using a *Caenorhabditis elegans* infection model. *Eukaryot Cell* **11**, 1750-1758.

Ratnappan, R., Amrit, F. R. G., Chen, S. -W., Gill, H., Holden, K., Ward, J. & Ghazi, A. (2014). Germline signals deploy NHR-49 to modulate fatty-acid β-oxidation and desaturation in somatic tissues of *Caenorhabditis elegans*. *PLoS Genetics* **10**, e1004829.

Rosenbach, A., Dignard, D., Pierce, J. V., Whiteway, M. & Kumamoto, C. A. (2010). Adaptations of *Candida albicans* for growth in the mammalian intestinal tract. *Eukaryot Cell* **9**, 1075–1086.

Saika, A., Nagatake, T. & Kunisawa, J. (2019). Host- and microbe-dependent dietary lipid metabolism in the control of allergy, inflammation, and immunity. *Front Nutr* **6**, 36.

Saville, S. P., Lazzell, A. L., Monteagudo, C. & Lopez-Ribot, J. L. (2003). Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell* **2**, 1053–1060.

Schaar, C. E., Dues, D. J., Spielbauer, K. K., Machiela, E., Cooper, J. F., Senchuk, M. *et al*. (2015). Mitochondrial and cytoplasmic ROS have opposing effects on lifespan. *PLoS Genet* **11**, e1004972.

Schwarz, D., Kisselev, P., Chernogolov, A., Schunck, W. -H. & Roots, I. (2005). Human CYP1A1 variants lead to differential eicosapentaenoic acid metabolite patterns. *Biochem Biophys Res Commun* **336**, 779–783

Schwarz, D., Kisselev, P., Ericksen, S. S., Szklarz, G. D., Chernogolov, A., Honeck, H., Schunck, W. -H. & Roots, I. (2004). Arachidonic and eicosapentaenoic acid metabolism by human CYP1A1: highly stereoselective formation of 17(R),18(S)-epoxyeicosatetraenoic acid. *Biochem Pharmacol* **67**, 1445–1457.

Serhan, C. N. (2017). Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J* **31**, 1273–88.

Shareck, J., Nantel, A. & Belhumeur, P. (2011). Conjugated linoleic acid inhibits hyphal growth in *Candida albicans* by modulating Ras1p cellular levels and downregulating *TEC1* expression. *Eukaryotic Cell* **10**, 565-577.

Sifri, C. D., Begun, J. & Ausubel, F. M. (2005). The worm has turned-microbial virulence modeled in *Caenorhabditis elegans*. *Trends Microbiol* **13**, 119-127.

Tampakakis, E., Okoli, I. & Mylonakis, E. (2008). A *C. elegans*-based, whole animal, *in vivo* screen for the identification of antifungal compounds. *Nat Protoc* **3**, 1925–1931.

Tao, J., Hao, Y., Li, X., Yin, H., Nie, X., Zhang, J., Xu, B., Chen, Q. & Li, B. (2020). Systematic identification of housekeeping genes possibly used as references in *Caenorhabditis elegans* by large-scale data integration. *Cells* **9** (3), 786.

Thibane, V. S., Ells, R., Hugo, A., Albertyn, J., van Rensburg, W. J., Van Wyk, P. W., Kock, J. L. & Pohl, C. H. (2012). Polyunsaturated fatty acids cause apoptosis in *C. albicans* and *C. dubliniensis* biofilms. *Biochim Biophys Acta* **1820**, 1463-1468.

Thomas, J. H. (2007). Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. *PLoS Genet* **3**, e67.

Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M. *et al*. (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* **2**, e183.

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* **3** (2), e53.

Vediyappan, G., Dumontet, V., Pelissier, F. & d'Enfert, C. (2013). Gymnemic acids inhibit hyphal growth and virulence in *Candida albicans*. *PLoS One* **8** (9), e74189.

Villa, S., Hamideh, M., Weinstock, A., Qasim, M. N., Hazbun, T. R., Sellam, A., Hernday. A. D. & Thangamani, S. (2020). Transcriptional control of hyphal morphogenesis in *Candida albicans*. *FEMS Yeast Res* **20** (1), foaa005.

Wagner, K., Inceoglu, B. & Hammock, B. D. (2011). Soluble epoxide hydrolase inhibition. epoxygenated fatty acids and nociception. *Prostaglandins Other Lipid Mediat* **96** (1-4), 76-83.

Wang, M., Chen, H., Gu, Z., Zhang, H., Chen, W. & Chen, Y. Q. (2013). ω-3 fatty acid desaturases from microorganisms: structure, function, evolution, and biotechnological use. *Appl Microbiol Biotechnol* **97** (24), 10255–62.

Wang, Y., Shi, J., Jiang, F., Xu, Y. J. & Liu, Y. (2022). Metabolomics reveals the impact of the saturation of dietary lipids on the aging and longevity of *C. elegans*. *Mol Omics* **18** (5), 430–438.

Watts, J. L. & Browse, J. (1999). Isolation and characterization of a Delta 5-fatty acid desaturase from *Caenorhabditis elegans. Arch Biochem Biophys* **362**, 175–182.

Watts, J. L. & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **99**, 5854–5859.

Wei, Y. & Kenyon, C. (2016). Roles for ROS and hydrogen sulfide in the longevity response to germline loss in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **113**, E2832–E2841.

Yoon, B. K., Jackman, J. A., Valle-Gonzáles, E. R. & Cho, N. -J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int J Mol Sci* **19**, E1114.

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X. & Liang, B. (2013). Comparative genomics and functional study of lipid metabolic genes in *Caenorhabditis elegans. BMC Genomics* **14**, 164.

Zhou, X. R., Green, A. G. & Singh, S. P. (2011). *Caenorhabditis elegans* ∆12- desaturase *fat-2* is a bifunctional desaturase able to desaturate a diverse range of fatty acid substrates at the ∆12 and ∆15 positions. *J Biol Chem* **286** (51), 43644–50.

.

Chapter 5

General discussions and conclusions

1. Introduction

The nematode *Caenorhabditis elegans* is readily infected with numerous human bacterial pathogens and amenable to various molecular tools, thus a reliable host model for understanding different facets of host-pathogen interaction, including various virulence factors and innate immunity pathways (Aballay and Ausubel, 2002; Moy *et al*., 2006; Breger *et al*., 2007; Durai *et al*., 2013; Kong *et al*., 2014). Despite numerous *in vitro* studies of polymicrobial interactions, the *in vivo* relevance of these interactions is still controversial, as different models of infection give contradictory results, suggesting the probable involvement of the immune system as a third-party player. Furthermore, the differential effect may be explained by the fact that many environmental factors are both different and dynamic between *in vitro* and *in vivo* conditions, including nutrient levels, microbe-substrate interactions, cell-cell interactions, secreted products from the host, immune pressure, and biophysical effects of the three-dimensional environment (Peleg *et al*., 2008). Because nematodes normally eat bacteria as their food source, they have an evolution that is shaped by interactions with both pathogenic and non-pathogenic microorganisms. Importantly, for protection against ingested pathogens, nematodes depends on inducible host defence mechanisms (Pukkila-Worley and Ausubel, 2012; Cohen and Troemel, 2015; Pukkila-Worley, 2016; Kim and Ewbank, 2018). These immune effectors include a suite of secreted proteins, such as lysozymes, proteins with CUB-like domains (for complement C1r/C1s, Uegf, Bmp1), and stichodactyla (ShK) toxins that required for host defence during bacterial infection (Troemel *et al*., 2006; Shapira *et al*., 2006; Nandakumar *et al*., 2008; Peterson *et al*., 2019). Indeed, *C*. *elegans* with mutations that abrogate the induction of these immune effectors during infection are hypersusceptible to killing by bacterial pathogens (Kim *et al*., 2002; Troemel *et al*., 2006; Shivers *et al*., 2010). Moreover, perturbations of the microbiome and a weakened host immune system are conditions that facilitates conversion of most opportunistic microbes from a commensal to a pathogenic state (Kim *et al*., 2002).

Caenorhabditis elegans is a powerful model organism to study the roles of polyunsaturated fatty acids (PUFAs) because it synthesizes all of the essential enzymes required to produce a range of omega-3 (n-3) and omega-6 (n-6) fatty acids (FAs), including an n-3 desaturase, an activity that is absent in most animals (Napier and Michaelson, 2001). Unlike mammals, *C. elegans* does not require essential FAs in its diet, but is capable of synthesising arachidonic acid (AA) and eicosapentaenoic acid (EPA), using only saturated and monounsaturated FAs from bacteria as precursors (Hutzell and Krusberg, 1982). Studies in *C. elegans* to date have mostly identified overlapping functions of 20-carbon n-3 and n-6 FAs in many biological

processes, such as reproduction and in neurons, and its ability to alter FA composition with dietary supplementation and genetic manipulation permits the dissection of these FAs functions, however, their specific roles in infections are beginning to emerge (Watts and Browse, 2002; Watts *et al*., 2003; Deline *et al*., 2013; Watts, 2016; Anderson *et al*., 2019). Since many microbes respond differently to a variety of natural and synthetic FAs, substantial efforts have been made to understand the unique features of FAs that function as antimicrobial compounds (Yoon *et al*., 2018). To understand the function of PUFAs, *C. elegans* has become a useful genetic model for evaluating the basic mechanisms that support the effects of specific PUFAs in a whole organism (Watts, 2016). This is important because despite n-6 and n-3 fatty PUFAs being structurally similar, their biological roles can be quite divergent. Numerous studies have shown the protective role of n-3 PUFA, EPA and docosahexaenoic acid (DHA), against cardiovascular and inflammatory/autoimmune diseases (Simopoulos, 2002; Oppedisano *et al*., 2020; Nienaber *et al*., 2020, 2022). For instance, these n-3 PUFAs have anti-inflammatory and pro-resolving properties, and it has been demonstrated that these n-3 PUFAs lower the excessive inflammation in tuberculosis (Nienaber *et al*., 2020, 2022). In this study, we uncovered the roles of AA and EPA supplementation as well as infection on the FA composition of *C. elegans*. We performed pathogenesis assay on PUFA-supplemented nematodes to test for pathogen survival. Our results showed that unlike AA, EPA supplementation inhibited *C. albicans* hyphal growth in the monomicrobial infected nematodes. Given the observed effect of EPA on *C. albicans* hyphal formation, we therefore, aimed to unravel the influence of EPA on *C. albicans* hyphal formation by conducting transcriptome analysis on *C. albicans* infected EPA supplemented nematodes, in order to identify differentially expressed genes under these conditions. To further investigate the effect of EPA, we treated *C. albicans* cells, both *in vitro* and *in vivo* using *C. elegans,* with 17,18-epoxyeicosatetraenoic acid [(17,18-EpETE; the most abundant cytochrome (CYP) eicosanoid in *C. elegans* (Kulas *et al*., 2008; Kosel *et al*., 2011)]. Lastly, we tested the hypothesis that inhibitors of mammalian EPA-metabolising CYP450 enzymes, 17-octadecynoic acid (17-ODYA) and 6-(2-propargyloxyphenyl)hexanoic acid (PPOH) has the ability to restore *C. albicans* hyphal formation *in vivo*.

2. Effect of polyunsaturated fatty acid supplementation and infection on *C. elegans* fatty acid composition and gene expression

Subsequent to intensive research, emerging evidence reveals that lipid metabolism influences the lifespan of numerous model organisms including *C. elegans* (Van Gilst *et al*., 2005; Taubert *et al*., 2006; Littlejohn *et al*., 2020). Moreover, *C*. *elegans* pathogenesis assays have defined requirements for specific lipids and lipogenesis enzymes in innate immune regulation and pathogen defence (Nandakumar and Tan, 2008; Kim *et al*., 2018; Anderson *et al*., 2019; Kumar *et al*., 2020; Nienaber *et al*., 2022). For instance, the Δ6-desaturase *fat-3*, involved in the synthesis of PUFAs, gamma-linoleic acid (GLA) and stearidonic acid (SDA), is needed for the basal expression of innate immune genes and resistance to infection by *P*. *aeruginosa* (Nandakumar and Tan, 2008). In addition, the 2 stearoyl-coenzyme A desaturases that synthesize the monounsaturated fatty acid (MUFA) oleic acid (OL) in *C*. *elegans*, *fat-6* and *fat-7*, are required for the induction of innate immune genes (Anderson *et al*., 2019). Despite numerous studies on the role of FA on infection, we still have limited understanding of the influence of PUFAs on infection of humans or other animal hosts. Therefore, to unravel this, we firstly studied the influence of *C. albicans* and *P. aeruginosa* infection on the FA composition of *C. elegans*. All infected nematodes showed changes in FA composition with a decrease in the unsaturation index compared to control. The monomicrobial infections of *C. elegans* displayed relatively subtle FA composition alterations, with *P. aeruginosa* infection showing more significance, while polymicrobial infections showed significant FA composition changes. Interestingly, most effects observed in the FA composition of co-infection reflected those caused by *P. aeruginosa* rather than *C. albicans*. A key finding of these studies was a remarkable significant decrease in EPA [the most predominant PUFA in all *C. elegans* (Watts and Browse, 2002)] during infections (Table 5.1). We therefore further determined the effect of EPA and AA supplementation on the FA composition of uninfected and infected nematodes. With both supplementations, we observed a significant decrease in the relative percentage of EPA, with a concomitant increase in the relative percentage linoleic acid (LA) and α-linolenic acid (ALA) compared to unsupplemented nematodes (Table 5.1). This was inconsistent with results observed by Bouyanfif (2019), where supplementation with EPA did not cause any significant change in EPA in wild-type nematodes, although a decreasing trend was seen for EPA as well as a non-significant increase in ALA similar to our results. In addition, EPA supplementation decreased the longevity of wild-type nematodes and elevated expression of oxidative stress genes, *oxi-1* and *mcp-1* (Bouyanfif, 2019). It was concluded that observed PUFA composition alterations in EPA supplemented nematodes may be attributed to either induced oxidative stress or to the effects of eicosanoids, produced by EPA via cyclooxygenase and P450 monooxygenase enzymes (Watts *et al*., 2003; Bouyanfif, 2019). In order to verify

these results, using EPA supplemented uninfected nematodes, we studied the relative expression of genes involved in FA metabolism and found that several genes, including *fat-2* and *cyp-29A3* were significantly up-regulated. *fat-2* encodes the Δ12 desaturase enzyme, which facilitates the biosynthesis of LA from its substrate, OL (Zhou *et al*., 2011; Wang *et al*., 2013). Therefore, this observed up-regulation of *fat-2* corresponds to the observed increase in the relative percentage of LA in EPA supplemented nematodes. In addition, it may explain the observed increase in ALA, as the FA metabolism is rerouted along this branch of the pathway. Furthermore, supplementation with EPA resulted in the up-regulation of *cyp-29A3*, which encodes one of the two major CYPs that oxidizes EPA to eicosanoids (Kulas *et al*., 2008), such as 17,18-epoxy eicosatetraenoic acid (17,18-EpETE).

Table 5.1. Effect of arachidonic acid (AA) and eicosapentaenoic acid (EPA) supplementation and infection on *C. elegans* fatty acid composition.

	***Candida albicans* infection**	***Pseudomonas aeruginosa* infection**	***C. albicans* and *P. aeruginosa* co-infection**
Unsupplemented nematodes	No change in composition	↓ EPA	↓ EPA ↑ oleic acid ↑ vaccenic acid ↑ α-linolenic acid
EPA supplemented nematodes	No change in composition	↓ EPA ↑ vaccenic acid ↓ linoleic acid ↓ oleic acid	↓ EPA ↑ vaccenic acid ↓ linoleic acid ↓ oleic acid
AA supplemented nematodes	↓ EPA	↓ EPA ↑ vaccenic acid ↓ linoleic acid ↓ oleic acid	↓ EPA ↑ vaccenic acid ↓ linoleic acid ↓ oleic acid

↑ represents increase and ↓ represents decrease in relative percentage composition

According to literature, dietary supplementation of OL as well as other MUFAs, including palmitoleic (POA) and *cis*-vaccenic acid (cVA), can extend the lifespan of unmated hermaphrodites (Han *et al*., 2017). Remarkably, OL treatment specifically restored the fat loss induced by mating, and also rescued the lifespan reduction induced by mating, without affecting reproduction (Han *et al*., 2017). However, supplementation of other FAs was not able to ameliorate the mating-induced death. In *C. elegans* and cell lines, dietary supplementation with n-6 PUFAs, AA and dihomo-gamma linolenic acid (DGLA), but not EPA activates autophagy, a pro-longevity process that promotes survival under nutrient deprivation (O'Rourke *et al*., 2013; Niso-Santano *et al*., 2015). In addition, it was reported that ingestion of fish oil can promote lipid peroxidation in different model animals, including *C. elegans*, and humans and induced shorter life span in *C. elegans* (Harats *et al*., 1991; Meydani *et al*., 1991; Kaasgaard *et al*., 1992; Miret *et al*., 2003; Tsuduki *et al*., 2011; Shmookler Reis *et al*., 2011; Sugawara *et al*., 2013; Bouyanfif, 2019). Authors believe that the reduction of lifespan by EPA may be attributed to the oxidative stress caused by the exposure of the nematodes to higher amounts of exogenous EPA in addition to regular physiological levels of endogenously synthesized FA required for *C. elegans* metabolism (Bouyanfif, 2019). Secondly, supplementation with either AA or EPA in mono- and polymicrobial infections resulted in changes in FA profiles. For instance, infection with *C. albicans* alone had minimal effect on the relative percentages of the AA- and EPA supplemented nematodes. However, the most significant changes in FA profiles were observed for infections by either *P. aeruginosa* alone, or in combination with *C. albicans*. These changes entailed significant decreases in relative percentage of EPA of *C. elegans*. Thus, more work was needed to elucidate the mechanisms and the role behind this decrease in EPA.

3. The role of polyunsaturated fatty acids on egg retention, susceptibility to infection and gene expression

To further examine *C. albicans-P. aeruginosa* interactions in infection and determine how the host environment influences outcome of co-infection, we adopted a *C. elegans* model to mimic the co-infections. As expected, monomicrobial infections by either *C. albicans* or *P. aeruginosa* reduced the survival of nematodes, compared to uninfected nematodes. The observed mortality of nematodes infected by *C. albicans* was associated with a filamentous invasion by this fungus through the cuticles of the nematodes, which serves as a strong predictive factor for mortality. On the other hand, the study revealed that *C. albicans-P. aeruginosa* co-infection leads to synergistic virulence and enhanced mortality compared to single infections by these

pathogens. Interestingly, no hyphal formation by *C. albicans* was observed in the co-infection. Thus, we can speculate that *P. aeruginosa* may play a role in hindering the formation of hyphae during co-infection (similar to *in vitro* results), while enhancing mortality of the nematodes. The synergistic virulence and reduced filamentous invasion of *C. albicans* in co-infections in this model were contrary to the well-studied antagonistic interactions of the two pathogens *in vitro* (Hughes and Kim, 1973; Bauernfeind *et al*., 1987; Hermann *et al*., 1999; de Macedo and Santos, 2005; Peleg *et al*., 2008). However, the enhanced virulence is consistent with numerous mouse *C. albicans*-bacterial co-infection models, including organotypic models, which result in enhanced virulence, cytokine production, and/or fungal invasion (Neely *et al*., 1986; Villar *et al*., 2005; Diaz *et al*., 2012; Xu *et al*., 2014; Nash *et al*., 2014, 2015; Bertolini *et al*., 2019). In addition, using a transparent juvenile zebrafish to model mucosal lung infection, Bergeron and co-workers (2017) showed that *C. albicans* and *P. aeruginosa* are synergistically virulent, resulting in enhanced mortality.

We next determined the effects of AA and EPA supplementation on the survival of nematodes with mono- and polymicrobial infection by *C. albicans* and *P. aeruginosa*. First, we tested the chemotaxis behaviour of *C. elegans* towards AA and EPA. Notably, this assay allows simultaneous assessment of a compound's toxicity towards *C. elegans*. We demonstrated that *C. elegans* does not display strong behavioural responses towards FAs, indicating that the nematodes do not avoid either AA or EPA in this scenario. Our infection results showed that PUFA-supplemented nematodes were significantly different from unsupplemented nematodes for pathogen survival. In monomicrobial infections, EPA supplementation modulated *C. albicans* pathogenesis by significantly extending the time needed to kill 50% of the nematodes, although it did not influence the time needed to kill 100% of the nematodes, compared to unsupplemented *C. albicans* infected nematodes (Table 5.2). Interestingly, control unsupplemented nematodes died mainly due to the invasive growth of *C. albicans* hyphae, especially during the first 48 hours of post infection. In contrast, EPA supplemented *C. elegans* harboured only *C. albicans* yeast cells in the gut (Table 5.2), suggesting that EPA supplementation inhibited the yeast to hyphal conversion and hyphal growth in the nematodes, and therefore prevented hyphal-mediated killing of the nematodes. According to literature, lipid molecules such as FAs and eicosanoids have been reported to modulate the *C. albicans* yeast to hyphal transition *in vitro* (McLain *et al*., 2000; Noverr and Huffnagle, 2004; Clement *et al*., 2007; Murzyn *et al*., 2010). For instance, FAs, including butyric acid (BA), capric acid (CA), lauric acid (LUA), myristic acid (MA), POA, OL, LA, conjugated linoleic acid, and AA, inhibited the yeast to hyphal transition induced under various conditions (McLain *et al*., 2000; Noverr and Huffnagle, 2004; Clement *et al*., 2007; Murzyn *et al*., 2010; Lee *et al.*, 2019; Prasath *et al*., 2019; Lee *et al*., 2020; Kim *et al.*, 2020; Kim *et al*., 2021). Additionally, lactobacilli

decreased *C. albicans* virulence via the inhibition of hyphal formation due to low pH, generated by the production of different short chain fatty acids (SCFAs), such as BA, by the lactobacilli (Buffo *et al*., 1984; Noverr and Huffnagle, 2004; De Barros *et al*., 2018). Furthermore, SCFAs inhibited the enzyme histone deacetylase in *C. albicans* which impaired fungal growth and morphogenesis (Nguyen *et al*., 2011; Latham *et al*., 2012).

Table 5.2. Effect of arachidonic acid (AA) and eicosapentaenoic acid (EPA) supplementation on *C*. *elegans* egg-retention and susceptibility to infection, relative to controls nematodes.

	***Candida albicans* infection**	***Pseudomonas aeruginosa* infection**	***C. albicans* and *P. aeruginosa* co-infection**
Unsupplemented nematodes	-Reduced survival -Presence of hyphae -More eggs retained	-Reduced survival -More eggs retained	-Reduced survival -No hyphal formation -More eggs retained
EPA supplemented nematodes	-Extended time to kill 50% nematodes -No hyphal formation -More eggs released	-Reduced survival -More eggs released	-Reduced survival -No hyphal formation -More eggs released
AA supplemented nematodes	-Reduced survival -Presence of hyphae -No changes in egg retention	-Extended time to kill 50% of nematodes -No changes in egg retention	-Extended time to kill 100% of nematodes -No hyphal formation -No changes in egg retention

In order to further elucidate the influence of EPA supplementation on *C. albicans* hyphal formation, we examined the influence of this PUFA on expression of *C. albicans* genes related to hyphal production and found many of them were up-regulated in supplemented nematodes. Specifically, among the 64 most highly expressed genes involved in hyphal formation, we identified *CAS5*, an important gene for both hyphal formation *in vivo* and the killing of *C. elegans*. Similar results were seen by Kuloyo and co-workers (2020), where the addition of the PUFA, AA, caused an up-regulation of genes involved in biofilm and hyphal formation, even though exposure to this PUFA inhibited morphogenesis. This was seen as a means to compensate for the hyphal inhibitory effect of AA. Interestingly, genes involved in hyphal formation that responded similarly to EPA *in vivo* and AA *in vitro* are *CRZ1*, *CTA4*, *ERG11*, *FCR1*, *SNQ2*, *TAC1*, *TEC1*, *YOR1* and *ZCF3*. Taken together, our study sheds new light on

how EPA disrupts *C. albicans* hyphal formation, as well as the differential response of *C. elegans* to EPA and *C. albicans*. We also observed that pathogenesis can also be influenced by the host immune response. In mammals, EPA is well known for its immunomodulatory effects via production of anti-inflammatory eicosanoids (Iwami *et al*., 2011; Szabó *et al*., 2020). In *C. elegans,* GLA and SDA are required for basal activity of the p38 MAP kinase pathway and immunity against *P. aeruginosa* (Nandakumar and Tan, 2008). Although these authors found that EPA is not involved in this, we examined the effect of EPA supplementation on expression of immune response genes during infection with *C. albicans*. Several genes involved in immune response, including *cyp-37B1*, *daf-16*, *fipr-22*, *ilys-2*, *lys-5*, *lys-6*, *spp-12* and *fat-3* were up-regulated. Interestingly, *cyp-37B1* is one of 12 genes identified by Pukkila-Worley and co-workers (2011) as a core immune response gene of *C. elegans* and up-regulation of *daf-16* by PUFAs was also observed previously Wang and co-workers (2022). Moreover, the observed up-regulation of *fat-3*, which produces the PUFAs, GLA and SDA, required for the basal expression of innate immune genes and resistance to infection by *P. aeruginosa* (Nandakumar and Tan, 2008), reveals that lipid metabolism is required for immune activation and pathogen defence. Furthermore, Anderson and co-workers (2019) revealed that *fat-6* and *fat-7* involved in synthesize of OL in *C. elegans*, are required for the induction of innate immune genes. Accordingly, nematodes with loss-of-function mutations in *fat-6* and *fat-7* are hypersusceptible to infection by diverse pathogens, which can be rescued by the addition of exogenous OL (Anderson *et al*., 2019). We also observed that *nhr-49*, which plays a role in regulating lipid metabolism and participates in a cytoprotective acute stress response program that functions parallel to and independently of SKN-1/Nrf2 and HLH-30/TFEB signalling (Lapierre *et al*., 2013; Blackwell *et al*., 2015; Goh *et al*., 2018), was also up-regulated. These results suggest that supplementation with EPA primes the immune response of *C. elegans*. It would be essential to demonstrate *in vitro* if these effectors are able to interact with non-pathogenic and pathogenic microbes and inhibit microbial growth or viability. Moreover, it is possible that in future other *C. elegans* gene families may be discovered that possess antimicrobial activity. It is additionally important to emphasize that differential expression after pathogen exposure does not suffice to prove an antimicrobial function of these genes. Such functional evidence needs to be obtained at the peptide or protein level. This may be achieved *in vivo* by silencing of the investigated gene. A common approach for this relies on RNA interference (RNAi) (Timmons *et al*., 2001). Another approach is to use knock-out mutants, which are already available for a large number of *C. elegans* genes and which can be produced through application of CRISPR/Cas technology (Consortium, 2012; Friedland *et al*., 2013; Dickinson *et al*., 2013).

Although EPA supplementation seemed to enhance survival of *C. elegans* infected with *C. albicans*, this was not the case for nematodes infected with *P. aeruginosa,* given that the nematodes were more susceptible to *P. aeruginosa*. Moreover, similar to EPA supplemented *P. aeruginosa* infected nematodes, we observed that EPA supplementation significantly reduced the survival of nematodes co-infected with both *P. aeruginosa* and *C. albicans* (Table 5.2). In contrast, supplementation of AA significantly extended the time needed to kill 50% of the nematodes but not to kill 100% of the nematodes compared to unsupplemented *P. aeruginosa* infected nematodes. Similarly, AA supplementation partially rescued the nematodes from co-infection of *C. albicans* and *P. aeruginosa* by extending the time to kill 100% of the nematodes (Table 5.2). Fatty acid supplementation is known to have diverse roles in pathogenic bacterial susceptibility (Nandakumar and Tan, 2008; Kim *et al*., 2018; Anderson *et al*., 2019; Kumar *et al*., 2020; Nienaber *et al*., 2022). For instance, previously, using the *C. elegans-P. aeruginosa* host-pathogen model, Nandakumar and Tan (2008) showed that GLA and SDA but not AA and EPA, are required for the basal expression of innate immune effectors and pathogen resistance in wild type *C*. *elegans*. Anderson and co-workers (2019) performed pathogenesis assays with *P*. *aeruginosa* in *C*. *elegans* and showed that OL is necessary for the induction of innate immune defences and for protection against bacterial pathogens. Similar roles of FAs can be seen in *Staphylococcus aureus* infections, where several FAs such as palmitic acid (PAL), OL, LA, eicosadienoic acid, docosadienoic acid and EPA at sub-minimum inhibitory concentration levels, inhibited biofilm formation and haemolytic activity in *S. aureus* (Kim *et al*., 2018). Additionally, herring oil, DHA and EPA were discovered to markedly extend the survival of *C. elegans* during infection with *S. aureus* (Kim *et al*., 2018). Moreover, palmetto oil, LUA and MA resulted in the reduction of *S. aureus* virulence in *C. elegans* and exhibited minimal cytotoxicity (Kim *et al*., 2018). Nienaber *et al*. (2020) showed that both EPA/DHA and iron independently administered to *Mycobacterium tuberculosis* infected mice, lowers inflammation and improves indices of anaemia during infection. Additionally, EPA/DHA lowered bacterial load, which may be related to the enhanced phagocytic ability of immune cells (Serhan *et al*., 2017; Nienaber *et al*., 2022) and increased synthesis of EPA and DHA-derived lipid mediators. However, more work is needed to determine the effects of PUFA supplementation on health span and lifespan after infection. As supplementation experiments have yielded different results so far, it will be important to compare the effects of dose and timing of supplementation with various FAs on longevity after infection.

The ability of *C. elegans* to lay eggs is usually influenced by numerous factors including environmental stressors such as availability of food, temperature, overcrowding, and

availability of sperm (Schafer, 2005). However, during unfavourable conditions, for instance lack of food or exposure to harmful food, the nematodes retain their eggs until the environment becomes more favourable (Corsi, 2015). Presumably this egg retention is a mechanism used by the nematodes to protect their progeny. Previously, Garsin and co-workers (2001) reported that exposure to pathogenic forms of *Enterococcus faecalis* can cause persistent intestinal infection and even death in *C. elegans* (Garsin *et al*., 2001). While other authors reported that exposure to other forms of pathogenic bacteria from the genus *Salmonella* and *Burkholderia* can affect egg retention, however these effects were not quantified (Aballay *et al*., 2000; O'Quinn *et al*., 2001). In our present study, to decipher the functional relationships between PUFAs and egg retention behaviour of infected nematodes, we used the egg-in-worm assay. We found that exposure to either or both *C. albicans* and *P. aeruginosa* causes a change in egg retention behaviour such that the degree of egg retention within a population of nematodes infected by these pathogens varied significantly from the control. Importantly, *C. elegans* exposed to both *C. albicans* and *P. aeruginosa* showed the highest level of egg retention compared to nematodes infected by either *C. albicans* or *P. aeruginosa* alone. This observed high egg retention in the unsupplemented co-infection, correlates with the increased virulence of the unsupplemented co-infection observed in the survival assay results, providing an indication that nematodes may attempt to protect their progeny during infection. Previous studies showed that some of the nematodes exposed to *Salmonella typhimurium* became laden with eggs, which hatched internally, suggesting that *S. typhimurium* may be affecting the egg laying process (Aballay *et al*., 2000). In addition, O'Quinn and co-workers (2001) reported that nematodes exposed to *Burkholderia pseudomallei* and *Burkholderia thailandensis* exhibited an egg laying-deficient phenotype. Majority of stage larva 4 nematodes exposed to *B. pseudomallei* or *B. thailandensis* became 'bags-of-worms' early in adulthood as a result of internal hatching of retained embryos (O'Quinn *et al*., 2001). Thus, suggesting that both species were able to inhibit egg laying in adults homozygous for a constitutive egg laying mutation (O'Quinn *et al*., 2001). We further investigated the effects of AA and EPA supplementation on egg retention of the infected nematodes. Surprisingly, we did not observe any significant difference in egg retention of both the uninfected and infected AA supplemented nematodes compared to unsupplemented nematodes (Table 5.2). However, a more striking effect for EPA supplementation was observed. Here there was a significant decrease in the egg retention of EPA supplemented nematodes infected with either or both *C. albicans* and *P. aeruginosa* compared to unsupplemented infected nematodes (Table 5.2). Therefore, our findings suggest that unlike AA, EPA supplemented nematodes were able to lay eggs efficiently.

Noteworthy, *C. elegans* has a simplified nervous system consisting of 302 neurons, and the network of neurons and their connections has been thoroughly mapped (White *et al*., 1986). An intact motor and neural circuit is necessary for the egg-laying process to be successful (Schafer, 2005)**.** Thus egg-laying behaviour has served as an important phenotypic assay for the genetic dissection of neuronal signal transduction mechanisms (Schafer, 2005). Diets deficient in n-3 and other PUFAs lead to defective neural function (Sinclair *et al*., 2007; Bazinet and Laye, 2014). For instance, the *fat-3* mutant strain, which lacks 20-carbon n-6 and n-3 PUFAs, shows uncoordinated movement and defective egg laying behaviour, phenotypes which are controlled by motor neurons and hermaphrodite-specific serotonergic vulva neurons (Lesa *et al*., 2003; Watts *et al*., 2003). Moreover, while 20-carbon n-6 and n-3 PUFAs are redundant in their ability to promote proper sperm migration to oocytes, a role for divergent activities of n-6 and n-3 PUFAs in reproduction is suggested by dietary studies. Watts and Browse (2006) discovered that dietary supplementation of the n-6 PUFA, DGLA resulted in sterility due to the destruction of germ cells. Supplementation of AA also led to germ cell death, although at a much higher concentration than DGLA. On the contrary, supplementation with n-3 FAs, such as EPA had no adverse effects on the nematodes (Watts and Browse, 2006). Therefore, we can speculate that the observed egg retention results obtained in this study may be influenced by many biological processes that occur in the host and is influenced by PUFA supplementation. Thus, further studies would be to consider the exact mechanism underlying our observations noted in this study by which the pathogens and PUFAs affect egg retention of the nematodes.

4. Influence of 17,18-epoxy eicosatetraenoic acid on hyphal formation *in vivo* and *in vivo*

Given the observed decrease in the relative percentage of EPA in the supplemented nematodes, and that EPA does not have the ability to affect hyphal formation *in vitro* (Willems *et al*., 2019), we hypothesised that it is unlikely that EPA directly affects *in vivo* hyphal production by *C. albicans,* but rather that its influence is indirect, possibly via *in vivo* produced eicosanoid products of EPA, such as 17,18-epoxy eicosatetraenoic acid (17,18-EpETE), which is the most abundant cytochrome (CYP) eicosanoid in *C. elegans* (Kulas *et al*., 2008; Kosel *et al*., 2011). Therefore, to further analyse the link between long chain PUFAs, CYP eicosanoids, and the observed *C. albicans* phenotype changes, we investigated 17,18-EpETE for its ability to inhibit hyphal formation both *in vitro* and *in vivo* in *C. elegans*. Firstly, we tested this hypothesis by exposing *C. albicans* to 17,18-EpETE in hyphal inducing media. This resulted in a reduction in germ tube formation in both media tested. Similarly, nematodes

exposed to 17,18-EpETE prior to infection also did not display any hyphae piercing the cuticle during *C. albicans* infection. Thus, revealing that 17,18-EpETE was able to inhibit hyphal formation of *C. albicans in vivo* (Figure 5.1A). This is similar to our *in vitro* results, however, the complete inhibition observed *in vivo* suggest that there might be additional or enhanced inhibitory compounds or processes in the nematodes. In order to confirm the involvement of CYP EPA metabolites, we added CYP inhibitors, 17-octadecynoic acid (17-ODYA) and 6-(2-propargyloxyphenyl)hexanoic acid (PPOH), to EPA supplemented infected nematodes. This resulted in rescue of hyphal formation (Figure 5.1B). Interestingly, previous studies have shown that AA-derived eicosanoids can stimulate the yeast to hyphal transition in *C. albicans in vitro* (Kalo-Klein and Witkin, 1990; Noverr and Huffnagle, 2004). This is the first report of an EPA-derived eicosanoid able to inhibit *C. albicans* filamentation *in vitro* and *in vivo*. So, this finding suggests that the major EPA-derived metabolite (17,18-EpETE) may contribute to the functional effects of EPA, acting as an anti-virulence agent against *C. albicans*. We cannot exclude the possibility that other EPA- and also AA-derived metabolites share the hyphal inhibition capacity of 17,18-EpETE.

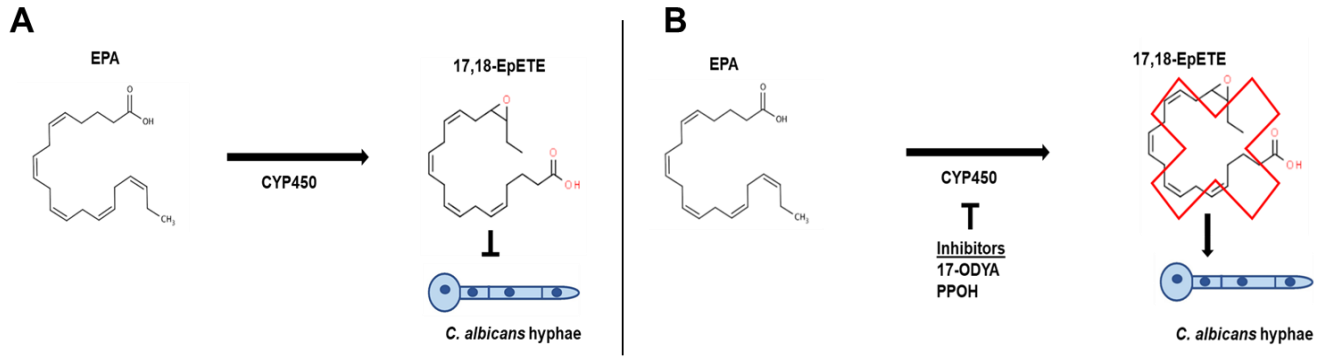


Figure 5.1. (A) Cytochrome P450 (CYP450) activity metabolises eicosapentaenoic acid (EPA) to 17,18-epoxy eicosatetraenoic acid (17,18-EpETE), which inhibits *Candida albicans* hyphal formation. (B) Cytochrome P450 inhibitors, 17-octadecynoic acid (17-ODYA) or 6-(2-propargyloxyphenyl)hexanoic acid (PPOH) inhibited the CYP450 to epoxidize EPA, thus EPA is not converted to 17,18-EpETE and *C. albicans* hyphal formation restored.

In this study it is clear that EPA had an effect on the survival of *C. albicans*-infected nematodes and on the hyphal formation. Yet, for future work, more experiments including transcriptome profiling, fitness profiling using collections of knock-out or over-expression mutants in *C.*

albicans, and target purification will be required to precisely decipher the targets of EPA. The identification of these EPA targets might lead to the discovery of additional inhibitors of fungal morphogenesis with broader application. Moreover, non-toxic small molecules such as FAs that are able to inhibit yeast to hyphal conversion and hyphal growth of *C. albicans* might result in us understanding the pathogenic fungal morphogenesis and may serve as templates for the development of novel antifungal agents. In conclusion, the rapid emergence of drug resistant microorganisms has driven the development of novel antifungals and antibiotics. This study shows that EPA has anti-hyphal activities in *C. albicans* and also reduce *C. albicans* virulence *in vivo* in *C. elegans*.

5. References

Aballay, A. & Ausubel, F. M. (2002). *Caenorhabditis elegans* as a host for the study of host-pathogen interactions. *Curr Opin Microbiol* **5**, 97–101.

Aballay, A., Yorgey, P. & Ausubel, F. A. (2000). *Salmonella typhmurium* proliferates and establishes a persistent infection in the intestine of *Caenorhabditis elegans. Curr Biol* **10**, 1539-1542.

Anderson, S. M., Cheesman, H. K., Peterson, N. D., Salisbury, J. E., Soukas, A. A. & Pukkila-Worley, R. (2019). The fatty acid oleate is required for innate immune activation and pathogen defence in *Caenorhabditis elegans*. *PLoS Pathogens* **15** (6).

Bauernfeind, A., Bertele, R. M., Harms, K., Horl, G., Jungwirth, R., Petermuller, C., Przyklenk, B. & Weisslein-Pfister, C. (1987). Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection* **15**, 270–277.

Bazinet, R. P. & Laye, S. (2014). Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nat Rev Neurosci* **15**, 771–785.

Bergeron, A. C., Seman, B. G., Hammond, J. H., Archambault, L. S., Hogan, D. A. & Wheeler, R. T. (2017). *Candida* and *Pseudomonas* interact to enhance virulence of mucosal infection in transparent zebrafish. *Infect Immun* **85**.

Bertolini, M., Ranjan, A., Thompson, A., Diaz, P. I., Sobue, T., Maas, K. & Dongari-Bagtzoglou, A. (2019). *Candida albicans* induces mucosal bacterial dysbiosis that promotes invasive infection. *PLoS pathogens* **15** (4), e1007717.

Blackwell, T. K., Steinbaugh, M. J., Hourihan, J. M., Ewald, C. Y. & Isik, M. (2015). SKN-1/Nrf, stress responses, and aging in *Caenorhabditis elegans. Free Radic Biol Med* **88**, 290–301.

Bouyanfif, A. (2019). Effects of fatty acid supplementation on gene expression, lifespan, and biochemical changes in wild type and mutant *C. elegans* strains. Texas Tech University, PhD Dissertation. https://hdl.handle.net/2346/85937

Breger, J., Fuchs, B. B., Aperis, G., Moy, T. I., Ausubel, F. M. & Mylonakis, E. (2007). Antifungal chemical compounds identified using a *Caenorhabditis elegans* pathogenicity assay. *PLoS Pathog* **3,** e18.

Buffo, J., Herman, M. A. & Soll, D. R. (1984). A characterization of pH-regulated dimorphism in *Candida albicans*. *Mycopathologia* **85**, 21–30.

Clement, M., Tremblay, J., Lange, M., Thibodeau, J. & Belhumeur, P. (2007). Whey-derived free fatty acids suppress the germination of *Candida albicans in vitro*. *FEMS Yeast Res* **7**, 276–285.

Cohen, L. B. & Troemel, E. R. (2015). Microbial pathogenesis and host defense in the nematode *C*. *elegans*. *Curr Opin Microbiol* **23**, 94–101.

Consortium CEDM. (2012). Large-scale screening for targeted knockouts in the *Caenorhabditis elegans* genome. *G3* (Bethesda) **2** (11), 1415–1425.

Corsi, A. K., Wightman, B. & Chalfie, M. (2015). A transparent window into biology: A primer on *Caenorhabditis elegans*. *Genetics* **200**, 387–407.

De Barros, P. P., Scorzoni, L., Ribeiro, F., Fugisaki, L. R., Fuchs, B. B., Mylonakis, E., Jorge, A. O., Junqueira, J. C. & Rossoni, R. D. (2018). *Lactobacillus paracasei* 28.4 reduces *in vitro* hyphae formation of *Candida albicans* and prevents the filamentation in an experimental model of *Caenorhabditis elegans*. *Microb Pathog* **117**, 80–87.

Deline, M., Keller, J., Rothe, M., Schunck, W. H., Menzel, R. & Watts, J. L. (2015). Epoxides derived from dietary dihomo-gamma-linolenic acid induce germ cell death in *C. elegans*. *Sci Rep* **5**, 15417.

de Macedo, J. L. & Santos, J. B. (2005). Bacterial and fungal colonization of burn wounds. *Mem Inst Oswaldo Cruz* **100**, 535–539.

Diaz, P. I., Xie, Z., Sobue, T., Thompson, A., Biyikoglu, B., Ricker, A., Ikonomou, L. & Dongari-Bagtzoglou, A. (2012). Synergistic interaction between *Candida albicans* and commensal oral streptococci in a novel *in vitro* mucosal model. *Infect Immun* **80** (2), 620–632.

Dickinson, D. J., Ward, J. D., Reiner, D. J. & Goldstein, B. (2013). Engineering the *Caenorhabditis elegans* genome using Cas9-triggered homologous recombination. *Nat Methods* **10**, 1028–1034.

Durai, S., Vigneshwari, L. & Balamurugan, K. (2013). *Caenorhabditis elegans*-based *in vivo* screening of bioactives from marine sponge-associated bacteria against *Vibrio alginolyticus*. *J Appl Microbiol* **115**, 1329–1342.

Friedland, A. E., Tzur, Y. B., Esvelt, K. M., Colaiácovo, M. P., Church, G. M & Calarco, J. A. (2013). Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. *Nat Methods* **10**, 741–743.

Garsin, D. A., Sifri, C. D., Mylonakis, E., Qin, X., Singh, K. V., Murray, B. E., Calderwood, S. B. & Ausubel, F. M. (2001). A simple model host for identifying Gram-positive virulence factors. *Proc Natl Acad Sci USA* **98**, 10892-10897.

Goh, G., Winter, J. J., Bhanshali, F., Doering, K., Lai, R., Lee, K., Veal, E. A. & Taubert, S. (2018). NHR-49/HNF4 integrates regulation of fatty acid metabolism with a protective transcriptional response to oxidative stress and fasting. *Aging cell* **17** (3), e12743.

Han, S., Schroeder, E. A., Silva-García, C. G., Hebestreit, K., Mair, W. B. & Brunet, A. (2017). Mono-unsaturated fatty acids link H3K4me3 modifiers to *C. elegans* lifespan. *Nature* **544**, 185–190.

Harats, D., Dabach, Y., Hollander, G., Ben-Naim, M., Schwartz, R., Berry, E. M., Stein, O. & Stein, Y. (1991). Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins. *Atherosclerosis* **90** (2-3), 127-139.

Hermann, C., Hermann, J., Munzel, U. & Rüchel, R. (1999). Bacterial flora accompanying *Candida* yeasts in clinical specimens. *Mycoses* **42** (11-12), 619–627.

Hughes, W. T. & Kim, H. K. (1973). Mycoflora in cystic fibrosis: some ecologic aspects of *Pseudomonas aeruginosa* and *Candida albicans*. *Mycopathol Mycol Appl* **50**, 261–269.

Hutzell, P. A. & Krusberg, L. R. (1982). Fatty acid compositions of *Caenorhabditis elegans* and *C. briggsae. Comp Biochem Physiol* **73B** (3), 517–520.

Iwami, D., Nonomura, K., Shirasugi, N. & Niimi, M. (2011). Immunomodulatory effects of eicosapentaenoic acid through induction of regulatory T cells. *International immunopharmacology* **11** (3), 384–389.

Kaasgaard, S. G., Holmer, G., Hoy, C. E., Behrens, W. A. & Beare-Rogers, J. L. (1992). Effects of dietary linseed oil and marine oil on lipid peroxidation in monkey liver *in vivo* and *in vitro*. *Lipids* **27** (10), 740-745.

Kalo-Klein, A. & Witkin, S. S. (1990). Prostaglandin E_2 enhances and gamma interferon inhibits germ tube formation in *Candida albicans*. *Infect Immun* **58** (1), 260–262.

Kim, D. H., Feinbaum, R., Alloing, G., Emerson, F. E., Garsin, D. A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M. W. & Ausubel, F. M. (2002). A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297,** 623-626.

Kim, D. H. & Ewbank, J. J. (2018). Signaling in the innate immune response. WormBook **2018**, 1–35.

Kim, Y. -G., Lee, J. -H., Park, J. G., & Lee, J. (2020). Inhibition of *Candida albicans* and *Staphylococcus aureus* biofilms by centipede oil and linoleic acid. *Biofouling* **36**, 126–137.

Kim, Y. -G., Lee, J. -H., Park, S., Kim, S. & Lee, J. (2021). Inhibition of polymicrobial biofilm formation by saw palmetto oil, lauric acid and myristic acid. *Microb Biotechnol* **00** (00), 1–13

Kim, Y. -G., Lee, J. -H., Raorane, C. J., Oh, S. T., Park, J. G. & Lee, J. (2018). Herring oil and omega fatty acids inhibit *Staphylococcus aureus* biofilm formation and virulence. *Front Microbiol* **9**, 1241.

Kong, C., Yehye, W. A., Abd Rahman, N., Tan, M. W. & Nathan, S. (2014). Discovery of potential anti-infectives against *Staphylococcus aureus* using a *Caenorhabditis elegans* infection model. *BMC Complement Altern Med* **14**, 4.

Kosel, M., Wild, W., Bell, A., Rothe, M., Lindschau, C. *et al*. (2011). Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of *Caenorhabditis elegans*. *Biochem J* **435**, 689–700.

Kulas, J., Schmidt, C., Rothe, M., Schunck, W. H. & Menzel, R. (2008). Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*. *Arch Biochem Biophys* **472**, 65–75.

Kuloyo, O., Fourie, R., Cason, E., Albertyn, J. & Pohl, C. H. (2020). Transcriptome analyses of *Candida albicans* biofilms, exposed to arachidonic acid and fluconazole, indicates potential drug targets. *G3* (*Bethesda)* **10** (9), 3099–3108.

Kumar, P., Lee, J. H., Beyenal, H. & Lee, J. (2020). Fatty acids as antibiofilm and antivirulence agents. *Trends Microbiol* **28** (9), 753–768.

Lapierre, L. R., De Magalhaes Filho, C. D., McQuary, P. R., Chu, C. -C., Visvikis, O., Chang, J. T. & Hansen, M. (2013). The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. *Nat Commun* **4**, 2267.

Latham, T., Mackay, L., Sproul, D., Karim, M., Culley, J., Harrison, D. J., Hayward, L., Langridge-Smith, P., Gilbert, N. & Ramsahoye, B. H. (2012) Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res* **40**, 4794–4803.

Lee, J. -H., Kim, Y. -G., Khadke, S. K. & Lee, J. (2020). Antibiofilm and antifungal activities of medium-chain fatty acids against *Candida albicans* via mimicking of the quorum-sensing molecule farnesol. *Microb Biotechnol* **14**, 1353–1366.

Lee, J. -H., Kim, Y. -G., Khadke, S. K., Yamano, A., Watanabe, A. & Lee, J. (2019). Inhibition of biofilm formation by *Candida albicans* and polymicrobial microorganisms by nepodin via hyphal-growth suppression. *ACS Infect Dis* **5**, 1177–1187.

Lesa, G. M., Palfreyman, M., Hall, D. H., Clandinin, M. T., Rudolph, C., Jorgensen, E. M. & Schiavo, G. (2003). Long chain polyunsaturated fatty acids are required for efficient neurotransmission in *C. elegans. J Cell Sci* **116**, 4965–4975.

Littlejohn, N. K., Seban, N., Liu, C. C. & Srinivasan, S. (2020). A feedback loop governs the relationship between lipid metabolism and longevity. *Elife* **9**, e58815.

McLain N., Ascanio R., Baker C., Strohaver R. A. & Dolan J. W. (2000). Undecylenic acid inhibits morphogenesis of *Candida albicans*. *Antimicrob Agents Chemother* **44**, 2873–2875.

Meydani, M., Natiello, F., Goldin, B., Free, N., Woods, M., Schaefer, E., Blumberg, J. B. & Gorbach, S. L. (1991). Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J Nutr* **121** (4), 484-491.

Miret, S., Saiz, M. P. & Mitjavila, M. T. (2003). Effects of fish oil- and olive oil-rich diets on iron metabolism and oxidative stress in the rat. *Br J Nutr* **89** (1), 11-18.

Moy, T. I., Ball, A. R., Anklesaria, Z., Casadei, G., Lewis, K. & Ausubel, F. M. (2006). Identification of novel antimicrobials using a live-animal infection model. *Proc Natl Acad Sci USA* **103**, 10414–10419.

Murzyn, A., Krasowska, A., Stefanowicz, P., Dziadkowiec, D. & Lukaszewicz, M. (2010). Capric acid secreted by *S. boulardii* inhibits *C. albicans* filamentous growth, adhesion and biofilm formation. *PLoS One* **5**, e12050.

Nandakumar, M. & Tan, M. W (2008). Gamma-linolenic and stearidonic acids are required for basal immunity in *Caenorhabditis elegans* through their effects on p38 MAP kinase activity. *PLoS Genet* **4** (11), e1000273.

Napier, J. & Michaelson, L. (2001). Genomic and functional characterization of polyunsaturated fatty acid biosynthesis in *Caenorhabditis elegans. Lipids* **36**, 761–766.

Nash, E. E., Peters, B. M., Fidel, P. L. & Noverr, M. C. (2015). Morphology-independent virulence of candida species during polymicrobial intra-abdominal infections with *Staphylococcus aureus*. *Infect Immun* **84**, 90–98.

Nash, E. E., Peters, B. M., Palmer, G. E., Fidel, P. L. & Noverr, M. C. (2014). Morphogenesis is not required for *Candida albicans-Staphylococcus aureus* intraabdominal infection-mediated dissemination and lethal sepsis. *Infect Immun* **82**, 3426–3435.

Neely, A. N., Law, E. J. & Holder, I. A. (1986). Increased susceptibility to lethal *Candida* infections in burned mice preinfected with *Pseudomonas aeruginosa* or pretreated with proteolytic enzymes. *Infect Immun* **52**, 200–204.

Nguyen, L. N., Lopes, L. C. L., Cordero, R. J. B. & Nosanchuk, J. D. (2011). Sodium butyrate inhibits pathogenic yeast growth and enhances the functions of macrophages. *J Antimicrob Chemother* **66**, 2573–2580.

Nienaber, A., Baumgartner, J., Dolman, R. C., Ozturk, M., Zandberg, L., Hayford, F., Brombacher, F., Blaauw, R., Parihar, S. P., Smuts, C. M. & Malan, L. (2020). Omega-3 Fatty acid and iron supplementation alone, but not in combination, lower inflammation and anemia of infection in *Mycobacterium tuberculosis*-infected mice. *Nutrients* **12** (9), 2897.

Nienaber, A., Ozturk, M., Dolman, R., Blaauw, R., Zandberg, L. L., van Rensburg, S., Britz, M., Hayford, F., Brombacher, F., Loots, D. T., Smuts, C. M., Parihar, S. P. & Malan, L. (2022). *n*-3 long-chain PUFA promote antibacterial and inflammation-resolving effects in *Mycobacterium tuberculosis*-infected C3HeB/FeJ mice, dependent on fatty acid status. *The Br J Nutr* **127** (3), 384–397.

Niso-Santano, M., Malik, S. A., Pietrocola, F., Bravo-San Pedro, J. M., Mariño, G., Cianfanelli, V., Ben-Younès, A., Troncoso, R., Markaki, M., Sica, V. *et al*. (2015). Unsaturated fatty acids induce non-canonical autophagy. *EMBO J* **34** (8), 1025-41.

Noverr, M. C. & Huffnagle, G. B. (2004). Regulation of *Candida albicans* morphogenesis by fatty acid metabolites. *Infect Immun* **72**, 6206–6210.

Oppedisano, F., Macrì, R., Gliozzi, M., Musolino, V., Carresi, C., Maiuolo, J., Bosco, F., Nucera, S., Caterina Zito, M., Guarnieri, L. *et al*. (2020). The anti-inflammatory and antioxidant properties of n-3 PUFAs: Their role in cardiovascular protection. *Biomedicines* **8** (9), 306.

O'Quinn, A. L., Wiegand, E. M. & Jeddeloh, J. A. (2001). *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin mediated paralysis. *Cell Microbiol* **3**, 381-393.

O'Rourke, E. J., Kuballa, P., Xavier, R. & Ruvkun, G. (2013). Omega-6 polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes Dev* **27**, 429–440.

Peleg, A. Y., Tampakakis, E., Fuchs, B. B., Eliopoulos, G. M., Moellering, R. C. *et al*. (2008). Prokaryote-eukaryote interactions identified by using *Caenorhabditis elegans. Proc Natl Acad Sci USA* **105**, 14585-14590.

Peterson, N. D., Cheesman, H. K., Liu, P., Anderson, S. M., Foster, K. J. *et al*. (2019). The nuclear hormone receptor NHR-86 controls anti-pathogen responses in *C. elegans*. *PLoS Genet* **15**, e1007935.

Prasath, K. G., Sethupathy, S. & Pandian, S. K. (2019). Proteomic analysis uncovers the modulation of ergosterol, sphingolipid and oxidative stress pathway by myristic acid impeding biofilm and virulence in *Candida albicans*. *J Proteomics* **208**, 103503.

Pukkila-Worley, R. & Ausubel, F. M. (2012). Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium. *Curr Opin Immunol* **24**, 3–9.

Pukkila-Worley, R., Ausubel, F. M. & Mylonakis, E. (2011). *Candida albicans* infection of *Caenorhabditis elegans* induces antifungal immune defences. *PLoS Pathog* **7** (6), e1002074.

Pukkila-Worley, R. (2016). Surveillance immunity: An emerging paradigm of innate defense activation in *Caenorhabditis elegans*. *PLoS Pathog* **12**, e1005795.

Schafer, W. R. (2005). Egg-laying, *WormBook*, ed. The *C. elegans* Research Community, WormBook.

Serhan, C. N., Chiang, N. & Dalli, J. (2017). New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. *Mol Asp Med* **64**, 1–17.

Shapira, M., Hamlin, B. J., Rong, J., Chen, K., Ronen, M. & Tan, M. W. (2006). A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc Natl Acad Sci USA* **103** (38), 14086-91.

Shivers, R. P., Pagano, D. J., Kooistra, T., Richardson, C. E., Reddy, K. C. *et al*. (2010). Phosphorylation of the conserved transcription factor ATF-7 by PMK-1 p38 MAPK regulates innate immunity in *Caenorhabditis elegans*. *PLoS Genet* **6**, e1000892.

Shmookler Reis, R. J., Xu, L., Lee, H., Chae, M., Thaden, J. J., Bharill, P., Tazearslan, C., Siegel, E., Alla, R., Zimniak, P. & Ayyadevara, S. (2011). Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging* (Albany NY) **3** (2), 125-147.

Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* **21** (6), 495–505.

Sinclair, A. J., Begg, D., Mathai, M. & Weisinger, R. S. (2007). Omega 3 fatty acids and the brain: Review of studies in depression. *Asia Pac J Clin Nutr* **16**, 391–397.

Sugawara, S., Honma, T., Ito, J., Kijima, R. & Tsuduki, T. (2013). Fish oil changes the lifespan of *Caenorhabditis elegans* via lipid peroxidation. *J Clin Biochem Nutr* **52**, 139–145.

Szabó, Z., Marosvölgyi, T., Szabó, É., Bai, P., Figler, M. & Verzár, Z. (2020). The potential beneficial effect of EPA and DHA supplementation managing cytokine storm in coronavirus disease. *Front Physiol* **11**, 752.

Taubert, S., Van Gilst, M. R., Hansen, M. & Yamamoto, K. R. A (2006). Mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in *C. elegans*. *Genes Dev* **20**, 1137–1149.

Timmons, L., Court, D. L. & Fire, A. (2001). Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. *Gene* **263**, 103–112.

Troemel, E. R., Chu, S. W., Reinke, V., Lee, S. S., Ausubel, F. M. & Kim, D. H. (2006). p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* **2** (11), e183.

Tsuduki, T., Honma, T., Nakagawa, K., Ikeda, I. & Miyazawa, T. (2011). Long-term intake of fish oil increases oxidative stress and decreases lifespan in senescenceaccelerated mice. *Nutrition* **27** (3), 334-337.

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* **3**, e53.

Villar, C. C., Kashleva, H., Mitchell, A. P. & Dongari-Bagtzoglou, A. (2005). Invasive phenotype of *Candida albicans* affects the host proinflammatory response to infection. *Infect Immun* **73**, 4588–4595.

Yoon, B. K., Jackman, J. A., Valle-González, E. R. & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. *Int J Mol Sci* **19**(4), 1114.

Wang, M., Chen, H., Gu, Z., Zhang, H., Chen, W. & Chen, Y. Q. (2013). ω-3 fatty acid desaturases from microorganisms: structure, function, evolution, and biotechnological use. *Appl Microbiol Biotechnol* **97** (24), 10255–62.

Wang, Y., Shi, J., Jiang, F., Xu, Y. J. & Liu, Y. (2022). Metabolomics reveals the impact of the saturation of dietary lipids on the aging and longevity of *C. elegans*. *Mol Omics* **18** (5), 430–438.

Watts, J. L. (2016). Using *Caenorhabditis elegans* to uncover conserved functions of omega-3 and omega-6 fatty acids. *J Clin Med* **5**, e19.

Watts, J. L., Phillips, E., Griffing, K. R. & Browse, J. (2003). Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in *Caenorhabditis elegans fat-3* mutants. *Genetics* **163**, 581–589.

Watts, J. L. & Browse, J. (2006). Dietary manipulation implicates lipid signaling in the regulation of germ cell maintenance in *C. elegans*. *Dev Biol* **292**, 381–392.

White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond Ser B Biol Sci* **314**, 1–340.

Willems, H., Stultz, J. S., Coltrane, M. E., Fortwendel, J. P. & Peters, B. M. (2019). Disparate *Candida albicans* biofilm formation in clinical lipid emulsions due to capric acid-mediated inhibition. *Antimicrob Agents Chemother* **63** (11), e01394-19.

Xu, H., Sobue, T., Thompson, A., Xie, Z., Poon, K., Ricker, A., Cervantes, J., Diaz, P. I. & Dongari-Bagtzoglou, A. (2014). Streptococcal co-infection augments *Candida* pathogenicity by amplifying the mucosal inflammatory response. *Cell Microbiol* **16,** 214–231.

Zhou, X. R., Green, A. G. & Singh, S. P. (2011). *Caenorhabditis elegans* ∆12- desaturase FAT-2 is a bifunctional desaturase able to desaturate a diverse range of fatty acid substrates at the ∆12 and ∆15 positions. *J Biol Chem* **286** (51), 43644–50.

Supplementary documents

1. **Supplementary Table S1.** *Caenorhabditis elegans* and *Candida albicans* genes used in this study

Caenorhabditis elegans		
Reference genes		
Probe Name	**Process/Description**	**References**
rps-2	Predicted structural constituent of ribosome	Tao *et al.* (2020) www.wormbase.org
rps-4	Predicted structural constituent of ribosome	Tao *et al.* (2020) www.wormbase.org
rps-23	Predicted structural constituent of ribosome	Tao *et al.* (2020) www.wormbase.org
Lipid metabolism		
Probe Name	**Process/Description**	**References**
cyp-29A2	Cytochrome P450, involved in lipid storage	Zhang *et al.* (2013) www.wormbase.org
cyp-29A3	Cytochrome P450, involved in eicosapentaenoic acid (EPA) metabolism	Liberman et al. (2020) www.wormbase.org
cyp-32A1	Cytochrome P450, involved in reproduction and possibly eicosanoid production	Benenati *et al.* (2009) www.wormbase.org
cyp-33C1	Cytochrome P450	www.wormbase.org
cyp-33E1	Cytochrome P450, orthologue of *cyp-33E2*, possibly involved in long-chain fatty acid metabolic process	www.wormbase.org
cyp-33E2	Cytochrome P450, involved in long chain fatty acid metabolic process and regulation of pharyngeal pumping	www.wormbase.org
cyp-37A1	Cytochrome P450, expressed in intestine, involved in lipid storage and life span	Zhang *et al.* (2013) www.wormbase.org
cyp-42A1	Cytochrome P450, involved in lipid metabolism and protection against glucotoxicity	Jung et al. (2020) www.wormbase.org
elo-1	Predicted fatty acid elongase, involved in unsaturated fatty acid biosynthetic process	www.wormbase.org
elo-2	Predicted fatty acid elongase, involved in several processes, including determination of adult lifespan, lipid metabolic process and reproduction	www.wormbase.org
elo-3	Predicted fatty acid elongase	www.wormbase.org
elo-4	Predicted fatty acid elongase	www.wormbase.org
elo-5	Predicted fatty acid elongase, expressed in amphid neurons and intestine	www.wormbase.org
elo-6	Predicted fatty acid elongase, expressed in amphid neurons, intestine, nerve ring and vulva	www.wormbase.org
elo-7	Predicted fatty acid elongase	www.wormbase.org
elo-8	Predicted fatty acid elongase	www.wormbase.org
elo-9	Predicted fatty acid elongase	www.wormbase.org
emb-8	NADPH-cytochrome P450 reductase, involved in eggshell formation and lipid biosynthetic process	www.wormbase.org
fat-1	Omega-3 fatty acid desaturase involved in positive regulation of locomotion and unsaturated fatty acid biosynthetic process	www.wormbase.org

fat-2	Delta 12-fatty acid dehydrogenase and stearoyl-CoA 9-desaturase involved in fatty acid biosynthetic process and innate immune response	www.wormbase.org
fat-3	Stearoyl-CoA 9-desaturase	www.wormbase.org
fat-4	Stearoyl-CoA 9-desaturase involved in unsaturated fatty acid biosynthetic process	www.wormbase.org
fat-5	Predicted to enable iron ion binding activity and stearoyl-CoA 9-desaturase activity, involved in long-chain fatty acid biosynthetic process	www.wormbase.org
fat-6	Stearoyl-CoA 9-desaturase activity, involved in innate immune response, long-chain fatty acid biosynthetic process and multicellular organism development	www.wormbase.org
fat-7	Stearoyl-CoA 9-desaturase activity, involved in fatty acid biosynthetic process	www.wormbase.org
mboa-7	Enables O-acyltransferase activity. Is involved in nematode larval development, oviposition and phosphatidylinositol biosynthetic process.	www.wormbase.org
nhr-49	Involved in determination of adult lifespan, positive regulation of transcription from RNA polymerase II promoter in response to stress, regulation of fatty acid metabolic process and regulates immuno-metabolic response to bacterial infection	Van Gilst *et al.* (2005) www.wormbase.org
Immune response		
Probe Name	**Process/Description**	**References**
abf-2	Involved in defence response to Gram-negative and Gram-positive bacteria	www.wormbase.org
abf-3	Involved in innate immune response to Gram-negative bacteria	www.wormbase.org
atf-7	Involved in several processes, including defence response to Gram-negative bacterium, regulation of innate immune response and regulation of transcription by RNA polymerase II	www.wormbase.org
cht-1	Chitinase involved in response to fungus	www.wormbase.org
clec-60	C-type lectin involved in defence response to Gram-positive bacteria, expressed in intestine	www.wormbase.org
clec-67	C-type lectin involved in PERK-mediated unfolded protein response. Is expressed in intestine	www.wormbase.org
cnc-2	Caenacin involved in defence response to Gram-negative bacterium, defence response to fungus and innate immune response, expressed in hypodermis	www.wormbase.org
cnc-4	Caenacin involved in defence response to fungus and innate immune response	www.wormbase.org
cnc-5	Caenacin involved in defence response to fungus and innate immune response	www.wormbase.org
cnc-6	Caenacin involved in defence response to fungus and innate immune response	www.wormbase.org
col-179	Predicted extracellular matrix structural constituent, involved in defence response to Gram-negative bacterium and innate immune response	www.wormbase.org

cyp-14A2	Cytochrome P450, expressed in intestine, possibly involved in stress response and detoxification	Thomas (2007) www.wormbase.org
cyp-37B1	Cytochrome P450, involved in defence response to Gram-positive bacteria	www.wormbase.org
daf-16	Involved in several processes, including defence response to other organisms	www.wormbase.org
fipr-22	Fungus induced peptide enriched in GABAergic neurons, excretory cell and hypodermis	www.wormbase.org
ilys-2	Lysozyme involved in defence response to Gram-positive bacterium	www.wormbase.org
ilys-5	Lysozyme	www.wormbase.org
lys-2	Lysozyme involved in defence response to Gram-negative and Gram-positive bacteria	www.wormbase.org
lys-4	Lysozyme involved in defence response to Gram-positive bacterium	www.wormbase.org
lys-5	Lysozyme involved in defence response to Gram-positive bacteria	www.wormbase.org
lys-6	Lysozyme	www.wormbase.org
lys-7	Lysozyme expressed in head neurons intestine and rectal gland cell	www.wormbase.org
spp-1	Caenopore involved in defence response to other organism and pore formation in membrane of another organism	www.wormbase.org
spp-2	Caenopore involved in defence response to Gram-positive bacteria	www.wormbase.org
spp-3	Caenopore involved in defence response to Gram-negative and Gram-positive bacteria	www.wormbase.org
spp-4	Caenopore enriched in hypodermis and intestine	www.wormbase.org
spp-8	Caenopore enriched in outer labial lateral neurons, polymodal nociceptive neurons and intestine	www.wormbase.org
spp-12	Caenopore involved in defence response to Gram-positive bacteria	www.wormbase.org
spp-14	Caenopore involved in immune response	www.wormbase.org
spp-23	Caenopore enriched in dorsal A neuron, ventral A neuron, intestine and pharyngeal muscle cell	www.wormbase.org
Candida albicans		
Reference genes		
Probe Name	**Process/Description**	**References**
ACT1	Actin	www.candidagenome.org Nailis *et al*. (2006)
LSC2	Putative succinate-CoA ligase beta subunit	www.candidagenome.org Nailis *et al*. (2006)
THD3	NAD-linked glyceraldehyde-3-phosphate dehydrogenase	www.candidagenome.org Nailis *et al*. (2006)
Filamentation		
Probe Name	**Process/Description**	**References**
ACE2 (orf19.6124)	Transcription factor that regulates morphogenesis, mutant is hyperfilamentous	www.candidagenome.org
ADA2 (orf19.2331)	Transcriptional coactivator, role in cell wall integrity. Mutation prevents hyphal formation in *Caenorhabditis elegans*	www.candidagenome.org

ADR1 (orf19.2752)	Transcription factor, transposon mutation affects filamentous growth	www.candidagenome.org
AFT2 (orf19.2272)	Putative transcription factor with role in hyphal growth	www.candidagenome.org
AHR1 (orf19.7381)	Transcription factor, involved in regulation of adhesion genes. Mutation affects hyphal growth	www.candidagenome.org
ALS1 (orf19.5741)	Cell-surface adhesin with role in biofilm formation and hyphal formation. Arachidonic acid (AA) responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
ALS3 (orf19.1816)	Cell wall adhesion and hyphal-associated invasin, mutation causes defect in biofilm formation	www.candidagenome.org
ASG1 (orf19.166)	Transcription factor, mutation causes decreased filamentation in certain strains	www.candidagenome.org
ASH1 (orf19.5343)	Transcription factor required for filamentous growth on solid media	www.candidagenome.org
ARG81 (orf19.4766)	Transcription factor, required for ornithine utilisation. Mutation may increase filamentation in certain strains.	www.candidagenome.org
BCR1 (orf19.723)	Transcription factor, regulates biofilm formation and involved in hyphal growth	www.candidagenome.org
BRE1 (orf19.976)	Putative E3 ubiquitin ligase, involved in ubiquitination of histone H2B during hyphal development, transposon mutation affects filamentous growth	www.candidagenome.org
BRG1 (orf19.4056)	Transcription factor, transposon mutation affects filamentation	www.candidagenome.org
CAS5 (orf19.4670)	Transcription factor, mutants have hyphal defect in *C. elegans* infection. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
CPH1 (orf19.4433)	Transcription factor involved in filamentation on solid media	www.candidagenome.org
CPH2 (orf19.1187)	Transcription factor, promotes hyphal growth	www.candidagenome.org
CRZ1 (orf19.7359)	Calcineurin-regulated transcription factor, mutation decreases hyphal formation in certain strains. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
CSR1 (orf19.3794)	Transcription factor, mutation affects filamentous growth	www.candidagenome.org
CTA4 (orf19.7374)	Transcription factor, involved in mating. Mutants may Mutations cause decreased filamentation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
CTA8 (*HSF1*) (orf19.4775)	Essential transcription factor, mutation may cause increased hyphal growth in certain strains	www.candidagenome.org
CUP9 (orf19.6514)	Transcription factor, mutants have increased filamentous growth. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
CWT1 (orf19.5849)	Transcription factor, mutation causes decreased filamentous growth in certain strains	www.candidagenome.org
CZF1 (orf19.3127)	Hyphal growth regulator. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
EFG1 (orf19.610)	Transcription factor, required for hyphal growth	www.candidagenome.org
EFH1 (orf19.5498)	Transcription factor, regulates filamentous growth	www.candidagenome.org

ERG11 (orf19.922)	Cytochrome P450 family, role in ergosterol biosynthesis and hyphal formation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
FCR1 (orf19.6817)	Transcription factor, transposon mutation enhances filamentation. Polyunsaturated fatty acid AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
FGR13 (orf19.1006)	Transposon mutation affects filamentous growth	www.candidagenome.org
FGR17 (orf19.5729)	Putative transcription factor, transposon mutation affects filamentous growth	www.candidagenome.org
FGR27 (orf19.6680)	Transcription factor, transposon mutation affects filamentous growth	www.candidagenome.org
FKH2 (orf19.5389)	Transcription factor, mutant lacks true hyphae, is constitutively pseudohyphal.	www.candidagenome.org
FLO8 (orf19.1093)	Transcription factor required for hyphal formation, regulates hyphal gene expression	www.candidagenome.org
GPR1 (orf19.1944)	Plasma membrane G-protein-coupled receptor required for wild type hyphal growth	www.candidagenome.org
GRF10 (orf19.4000)	Putative transcription factor, involved in control of filamentous growth	www.candidagenome.org
HAP5 (orf19.1973)	Transcription factor with roles in filamentous growth	www.candidagenome.org
HMS1 (orf19.921)	Transcript factor required for morphogenesis induced by elevated temperature	www.candidagenome.org
HOT1 (orf19.3328)	Required for inhibition of filamentous growth by farnesoic acid, filament induced	www.candidagenome.org
HWP1 (orf19.13.21)	Hyphal cell wall protein	www.candidagenome.org
MED7 (orf19.232)	Subunit of the RNA polymerase II mediator complex. Mutations may affect filamentation in certain strains	www.candidagenome.org
MSS11 (orf19.6309)	Transcription factor required for hyphal growth	www.candidagenome.org
NDT80 (orf19.2119)	Activator of *CDR1* induction by antifungal drugs, required for Spider biofilm formation and hyphal formation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
NGS1 (orf19.7516)	Acts as N-acetylglucosamine (GlcNAc) sensor required for GlcNAc-induced filamentation	www.candidagenome.org
NOT3 (orf19.2012)	Transcriptional regulator, mutant colonies exhibit slightly decreased filamentation ratio	www.candidagenome.org
NOT5 (orf19.5107)	Member of the transcription regulatory CCR4-NOT complex, required for hyphal growth	www.candidagenome.org
NRG1 (orf19.7150)	Transcription factor/repressor, regulates hyphal gene induction. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
OFI1 (orf19.4972)	Putative transcription factor involved in regulation of filamentous growth	www.candidagenome.org
OPI1 (orf19.1543)	Transcription factor, involved in regulation of filamentous growth	www.candidagenome.org
PGA13 (orf19.6420)	GPI-anchored cell wall protein involved in cell wall synthesis. Mutation may cause delayed filamentation in certain strains. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
PHO4 (orf19.1253)	Transcription factor, required for phosphate acquisition and stress resistance. Mutations may	www.candidagenome.org

	cause increased filamentous growth in certain strains	
PPR1 (orf19.3986)	Transcription factor, mutants have decreased hyphal growth in certain strains	www.candidagenome.org
RBF1 (orf19.5558)	Transcription factor, mutation causes accelerated induction of filamentous growth	www.candidagenome.org
RCA1 (orf19.6102)	Protein involved in regulation of carbonic anhydrases. mutation affects filamentous growth	www.candidagenome.org
RFG1 (orf19.2823)	Transcriptional repressor of filamentous growth and hyphal genes. Possible AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
RFX2 (orf19.4590)	Transcriptional repressor, regulator of filamentation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
RIM101 (orf19.7247)	Transcription factor, required for alkaline-induced hyphal growth. Possible AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
RLM1 (orf19.4662)	Transcription factor, mutation causes decreased hyphal growth on lactate	www.candidagenome.org
ROB1 (orf19.4998)	Transcription factor, mutant displays abnormal colony morphology (no peripheral hyphae) and invasive growth. Possible AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
RON1 (orf19.513)	Required for GlcNAc-induced hyphal growth	www.candidagenome.org
RTG3 (orf19.2315)	Transcription factor, mutation causes decreased filamentous growth in certain strains	www.candidagenome.org
SAP6 (orf19.5542)	Expressed during hyphal growth, involved in biofilm formation, AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SEF1 (orf19.3753)	Transcription factor, regulates iron uptake. Mutation may decrease filamentation in certain strains.	www.candidagenome.org
SET3 (orf19.7221)	NAD-dependent histone deacetylase, mutations affect filamentous growth. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SFL1 (orf19.454)	Transcription factor involved in negative regulation of morphogenesis. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SFL2 (orf19.3969)	Transcription factor involved in regulation of morphogenesis, required for filamentous growth	www.candidagenome.org
SIN3 (orf19.6011)	Transposon mutation affects filamentous growth	www.candidagenome.org
SKN7 (orf19.971)	Predicted response regulator protein. Mutation causes absence of hyphal formation in certain strains	www.candidagenome.org
SKO1 (orf19.1032)	Transcription factor, represses the yeast-to-hypha transition	www.candidagenome.org
SMI1 (orf19.5058)	Cell wall biosynthesis protein, Cyr1-induced in hyphal cells	www.candidagenome.org
SNF4 (orf19.5768)	Putative subunit of the AMP-activated Snf1p kinase, transposon mutation affects filamentation	www.candidagenome.org
SNF5 (orf19.5871)	Part of SWI/SNF complex which is essential for hyphal growth	www.candidagenome.org
SNF6 (orf19.831)	Part of SWI/SNF complex which is essential for hyphal growth	www.candidagenome.org
SNQ2 (orf19.5759)	Transporter, transposon mutation affects filamentation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SPT3 (orf19.7622)	Homozygous null mutant is hyperfilamentous	www.candidagenome.org

SPT6 (orf19.7136)	Putative transcription elongation factor, transposon mutation affects filamentous growth	www.candidagenome.org
SPT20 (orf19.422)	Putative transcription factor, mutants have decreased hyphal and invasive growth	www.candidagenome.org
SSN6 (orf19.6798)	Hyphal growth regulator, repressed during hyphal growth	www.candidagenome.org
STD1 (orf19.6173)	Putative transcription factor, mutation causes increased filamentation	www.candidagenome.org
STP2 (orf19.4961)	Amino-acid-regulated transcription factor. Mutation decreases filamentation	www.candidagenome.org
SWI1 (orf19.5657)	Part of SWI/SNF complex which is essential for hyphal growth	www.candidagenome.org
SWI4 (orf19.4545)	Putative component of the SBF transcription complex involved in G1/S cell-cycle progression, mutants have reduced peripheral filamentation	www.candidagenome.org
TAC1 (orf19.3188)	Zn(2)-Cys(6) transcriptional activator of drug-responsive genes, mutation causes decreased hyphal formation in certain strains. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
TCC1 (orf19.6734)	Putative transcription factor, regulation of filamentation and expression of hypha-specific genes	www.candidagenome.org
TEA1 (orf19.6985)	Putative transcription factor, mutation may cause increased hyphal growth in certain strains	www.candidagenome.org
TEC1 (orf19.5908)	Transcription factor involved in hyphal gene regulation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
TFG1 (orf19.4585)	Transposon mutation affects filamentous growth	www.candidagenome.org
TUP1 (orf19.6109)	Transcriptional corepressor, represses filamentous growth, role in germ tube induction	www.candidagenome.org
UME6 (orf19.1822)	Transcription factor that regulates filamentous growth	www.candidagenome.org
WOR1 (orf19.4884)	Transcription factor ("master switch") of white-opaque phenotypic switching. Mutants have decreased biofilm formation in certain strains	www.candidagenome.org
YOR1 (orf19.1783)	ABC-type plasma membrane transporter involved in resistance to aureobasidin A. Mutation causes a decrease in invasive growth. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
ZCF3 (orf19.1168)	Transcription factor required for filamentous growth	www.candidagenome.org
ZCF7 (orf19.1685)	Predicted transcription factor, mutation causes decreased colony wrinkling and hyphal formation.	www.candidagenome.org
ZCF11 (orf19.2423)	Transcription factor required for wild-type filamentous growth	www.candidagenome.org
ZCF14 (orf19.2647)	Putative transcription factor, mutants have decreased hyphal growth	www.candidagenome.org
ZCF17 (orf19.3305)	Putative transcription factor, mutants have increased hyphal growth, but decreased invasive growth	www.candidagenome.org
ZCF18 (orf19.3405)	Putative transcription factor, mutants have increased hyphal growth	www.candidagenome.org
ZCF29 (orf19.5133)	Transcription factor, mutants have defects in filamentous growth	www.candidagenome.org

ZCF32 (orf19.5940)	Transcription factor involved in regulation of biofilm formation	www.candidagenome.org
ZNC1 (orf19.3187)	Transcription factor required for adherence and represses hyphal cell formation	www.candidagenome.org
	Other processes	
Probe Name	**Process/Description**	**References**
CDR1 (orf19.6000)	Multidrug transporter of ABC superfamily, transport phospholipids. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
CDR2 (orf19.5958)	Multidrug transporter, ATP-binding cassette (ABC) superfamily, transports phospholipids. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
DAL8 (orf19.5859)	Putative allantoate permease, fungal-specific, AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
EHT1 (orf19.3040)	Putative acyl-coenzymeA: ethanol O-acyltransferase. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
ECM17 (orf19.4099)	Putative sulfite reductase beta subunit, role in cell wall biogenesis. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
FAH2 orf19.2184	Putative fumarylacetoacetate hydrolase	www.candidagenome.org
FLU1 (orf19.6577)	Multidrug efflux pump of the plasma membrane AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
HRD3 orf19.1191	Ortholog(s) have ubiquitin-protein transferase activity and role in negative regulation of protein autoubiquitination	www.candidagenome.org
HSP12 (orf19.3160)	Decreased expression in hyphae. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
IPT1 (orf19.4769)	Inositol phosphoryl transferase, catalyzes the synthesis of the most abundant sphingolipid. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
MET4 (orf19.5312)	Putative transcription coactivator, predicted role in sulphur amino acid metabolism. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
MDR1 (orf19.5604)	Plasma membrane MDR/MFS multidrug efflux pump. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
PDR16 (orf19.1027)	Phosphatidylinositol transfer protein. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
PST1 (orf19.2241)	Hyphal-induced. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
RGT1 (orf19.4722)	Transcription factor, transcriptional repressor involved in the regulation of glucose transporter genes	www.candidagenome.org
RTA3 (orf19.23)	7-transmembrane receptor protein involved in regulation of asymmetric lipid distribution in plasma membrane. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SUL2 (orf19.2738)	Putative sulfate transporter. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
SUT1 (orf19.4342)	Transcription factor involved in sterol uptake. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
TYE7 (orf19.4941)	Transcription factor, control of glycolysis. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)
UGA3 (orf19.7570)	Transcription factor, required for utilization of gamma-aminobutyrate	www.candidagenome.org

UPC2 (orf19.391)	Transcription factor, regulates ergosterol biosynthesis	www.candidagenome.org
YWP1 (orf19.3618)	Secreted yeast wall protein, involved in adhesion and biofilm formation. AA responsive gene	www.candidagenome.org Kuloyo *et al*. (2020)

2. References

Benenati, G., Penkov, S., Müller-Reichert, T., Entchev, E. V. & Kurzchalia, T. V. (2009). Two cytochrome P450s in *Caenorhabditis elegans* are essential for the organization of eggshell, correct execution of meiosis and the polarization of embryo. *Mech Dev* **126** (5-6), 382–393.

Candida Genome Database. http://72/

Jung, Y., Kwon, S., Ham, S., Lee, D., Park, H. H., Yamaoka, Y., Jeong, D. E., Artan, M., Altintas, O., Park, S. *et al*. (2020). *Caenorhabditis elegans* Lipin 1 moderates the lifespan-shortening effects of dietary glucose by maintaining ω-6 polyunsaturated fatty acids. *Aging cell* **19** (6), e13150.

Liberman, N., O'Brown, Z. K., Earl, A. S., Boulias, K., Gerashchenko, M. V., Wang, S. Y., Fritsche, C., Fady, P. E., Dong, A., Gladyshev, V. N. & Greer, E. L. (2020). N6-adenosine methylation of ribosomal RNA affects lipid oxidation and stress resistance. *Sci Adv* **6** (17), eaaz4370.

Kuloyo, O., Fourie, R., Cason, E., Albertyn, J. & Pohl, C. H. (2020). Transcriptome analyses of *Candida albicans* biofilms, exposed to arachidonic acid and fluconazole, indicates potential drug targets. *G3* (*Bethesda)* **10** (9), 3099–3108.

Nailis, H., Coenye, T., Van Nieuwerburgh, F., Deforce, D. & Nelis, H. J. (2006). Development and evaluation of different normalization strategies for gene expression studies in *Candida albicans* biofilms by real-time PCR. *BMC Mol Biol* **7**, 25.

Tao, J., Hao, Y., Li, X., Yin, H., Nie, X., Zhang, J., Xu, B., Chen, Q. & Li, B. (2020). Systematic identification of housekeeping genes possibly used as references in *Caenorhabditis elegans* by large-scale data integration. *Cells* **9** (3), 786.

Thomas, J. H. (2007). Rapid birth-death evolution specific to xenobiotic cytochrome P450 genes in vertebrates. *PLoS Genet* **3**, e67.

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*. *PLoS Biol* **3** (2), e53.

WormBase. https://wormbase.org/#012-34-5

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X. & Liang, B. (2013). Comparative genomics and functional study of lipid metabolic genes in *Caenorhabditis elegans. BMC Genomics* **14**, 164.