

## Tug-of-war between host immunity and microbial pathogenesis using *Caenorhabditis elegans* as a model system

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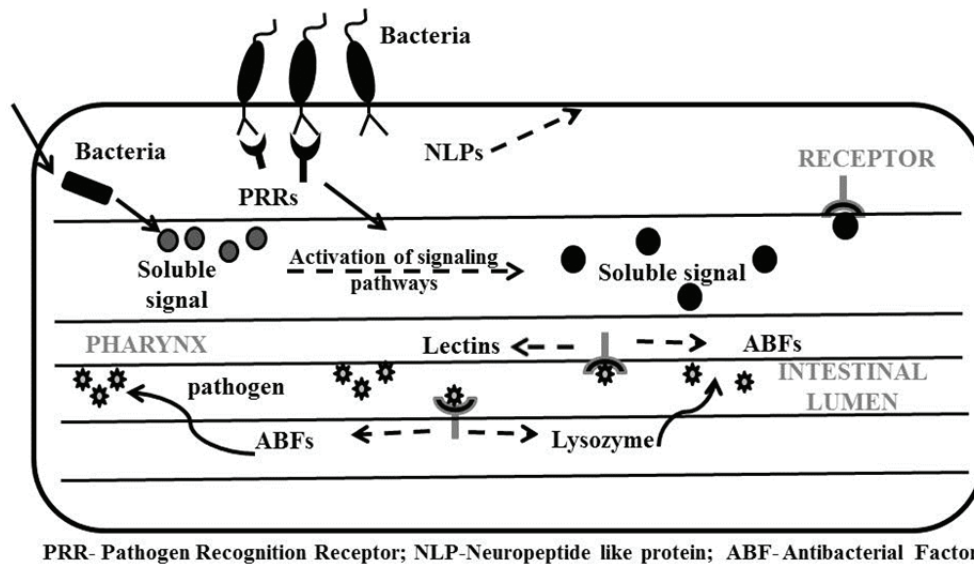
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Globally, bacterial infections have been recognized as a predominant issue associated with significant morbidity and mortality. This global burden could be overcome by understanding the mechanism of both microbial virulence and host immune response. One approach to identify novel virulence strategies adopted by pathogens and host immune defense is to study the host-pathogen interaction using suitable model system which could be an analogue for human. Testing pathogenesis and immune defense in rodent is generally not feasible due to its expensive maintenance. Therefore, the invertebrate nematode model *Caenorhabditis elegans* could represent a valuable alternative. The era of post-genomics has set a milestone in research by identifying and validating an appropriate potential human “disease causing” or “disease associated” genes by using host model system. Consequently, investigation of host-pathogen interaction has always been spotlighted area to the scientific community that decodes the mechanism of bacterial virulence including the conserved system of host defense and antimicrobial factors like c-type lectins, lysozymes, neuropeptide-like protein, etc. In this review, we present the host-pathogen interaction using *C. elegans* as an appropriate model to decipher the alteration happened at the context of both host and pathogen. Infection in *C. elegans* by microbes induces a number of defensive mechanisms which are evolutionary conserved to mammalian innate immunity. Additionally, it has also been exhibited that several microbial virulence factors required for complete pathogenicity in mammals are indeed required for causing infection in nematodes. Based on these facts, a number of novel virulence strategies adopted by pathogens as well as conserved hosts’ immune defense against respective infections have been screened using *C. elegans* which opens new avenue to develop novel alternate therapeutics.

**Keywords:** *Caenorhabditis elegans*; Microbial pathogenesis; Host defense; Innate immunity

### 1. Introduction

Infectious diseases have been recognized as one of the global burden that largely affects the world economy. To face this global crisis by infectious diseases, the interaction between host and infectious agents has required to be understood. Host-pathogen interaction is one such dynamic approach that equally reveals the hard wired mechanism of host immune response and microbial virulence mechanism underlie the human diseases [1]. Moreover, the post-genomic era has set a mile stone in research by identifying and validating the appropriate potential “disease-causing” or “disease-associated” genes using model organisms. This conferred an idea on mechanism of respective microbial infection and host immune defense that facilitates us to effectively cure or prevent the infection caused by pathogens. On the other hand, to combat the host immune defense bacterial pathogens have wisely added a number of virulence factors to cause a persistent infection in host. Hence, diverse vertebrate and non-vertebrate model organisms including mice, rat, *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, *Drosophila melanogaster*, Zebra fish, Arabidopsis etc., have been employed to screen the virulence factors and decipher the common virulence mechanism shares from evolutionarily higher hosts to lower hosts by pathogens [2,3]. This signifies that the crucial and critical steps needed to be identified in host-pathogen interaction are both bacterial virulence mechanisms as well as host defense systems. To counteract this multifaceted enigma, for the past 10 years, a most suitable model organism like *C. elegans* which is highly comparable to the mammals have been employed to decipher the host-pathogen interactions (Fig. 1). Here we reviewed how the nematode *C. elegans* has been utilized to provide insights into the response of both host and the pathogen that underlie the host-pathogen interface.



**Fig. 1** The schematic diagram showing the host defense against bacterial invasion. The invaded bacteria are detected by PRRs present in cell and that subsequently induced signals to produce specific antimicrobial peptide(s) that eliminate or kill the bacteria by inducing cell damage in invaded microbes.

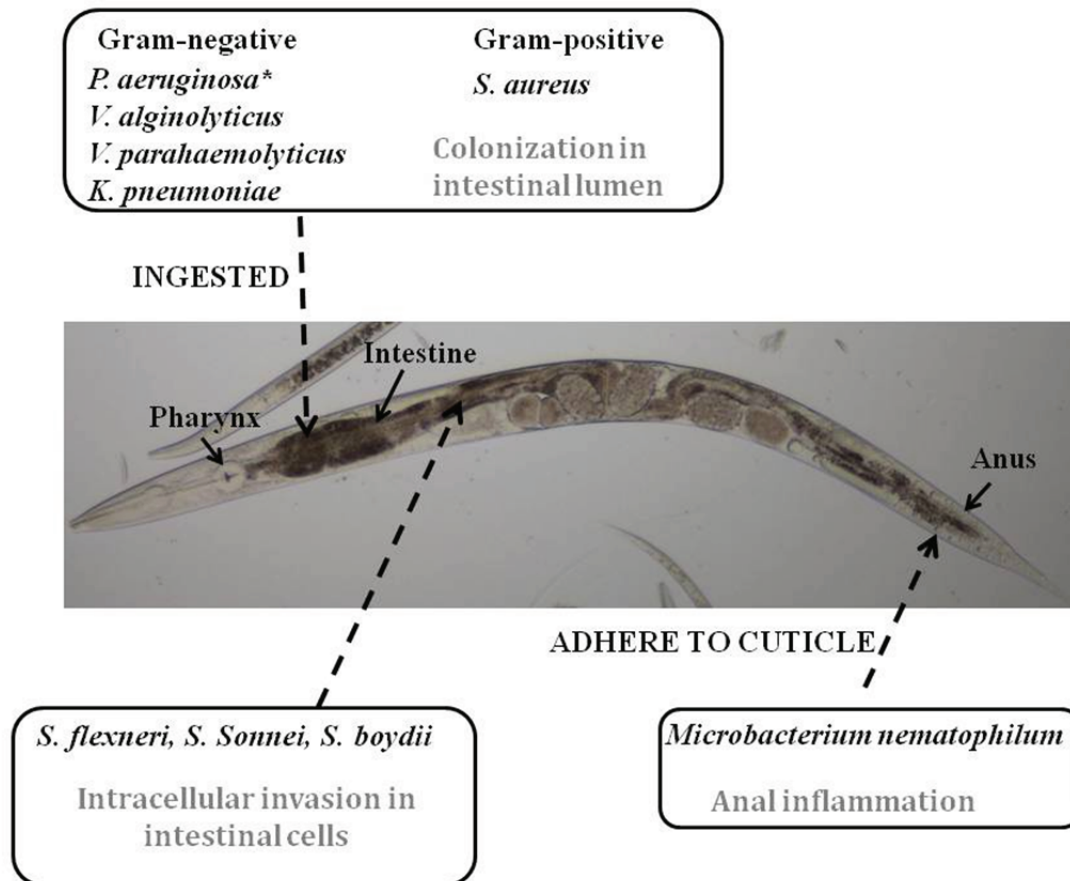
## 2. *Caenorhabditis elegans*, a model for host-pathogen interaction

*C. elegans* is a free-living soil nematode that feeds on bacteria and therefore it constantly exposed to various pathogens. For this reason, *C. elegans* has been positively exploited to investigate the virulence mechanism adopted by the human pathogens [4,5]. Additionally, the other experimental advantages associated with simple growth conditions, transparent body, known sequence, rapid generation time (3 days), short body length (1.5mm adults), large brood size (~300 progeny per nematode), short lifespan (~3 weeks) and completely sequenced genome (~100Mbp) makes the model organism more amenable for the study of developmental genetics, toxicology, neurobiology, systems biology and ageing. The unique transparent body nature of the nematodes under microscope has been allowed researcher to observe many biological processes like organogenesis, behavior, cell division/lineage and most importantly the bacterial pathogenesis. The over expression studies of human proteins or genes in *C. elegans* using cell- or tissue specific promoters or studying the differential gene expression profile of worms at proteomic and transcriptomic level by 2D gel electrophoresis and microarray analysis decipher the altered regulation in infected host at their molecular level. This reveals the identification of responsible signaling cascades that are elicited during diseases and that pave the way for designing drug targets for different therapies. Another modern approach known as double stranded RNA interference (dsRNAi) has also been established using *C. elegans* as a model to screen the gene(s) responsible for various diseases.

Hitherto, there is an ever-growing number of clinically significant Gram-positive and Gram-negative bacteria have been recognized to infect *C. elegans* [5-7]. An opportunistic human pathogen *Pseudomonas aeruginosa* is the first microorganism has been identified to infect and cause lethal effects in *C. elegans*. Additionally, it has been utilized to identify the bacterial genes required for the complete virulence under *in vivo* conditions in the nematode was later found to be necessary in other model organism [8]. Several studies have demonstrated that the virulence factors required for the mortality in *C. elegans* were also required for pathogenesis in mammals. This opens a new avenue for the development of novel approaches that target specific virulence mechanisms.

## 3. Route of infection by bacterial pathogens

Generally, pathogens of *C. elegans* used pharynx and epidermis as their two main routes of infection (Fig. 1). Most of the well noted Gram-positive and Gram-negative bacteria infected worms by oral feeding and established a persistent intestinal infection. In several cases, it has been shown that the ingested bacteria destroy the pharyngeal grinder and intestinal cells [9-11]. Certain bacterial pathogenic bacteria could adhere to the surface of the body and infect the nematodes [12,13]. Subsequently these bacterial infections provoke the innate immune response of host.



**Fig. 2** Bacterial pathogens of *C. elegans* and their mode of infections. Virtually all known bacterial pathogens are ingested, proliferated and establish an infection in the intestinal region of the nematode. Certain bacteria (\*) secreted toxins that could cause mortality in *C. elegans*. Only few of the known bacterial pathogens of *C. elegans* are included in the schematic diagram.

#### 4. *C. elegans* as a model for bacterial infection

In this review, the host response of *C. elegans* against bacterial pathogens (*Cronobacter sakazakii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Shigella boydii*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus* and *Vibrio parahaemolyticus*) and modifications in lipopolysaccharides (LPS) of pathogens and its virulence factor responsible for nematode killing have been discussed.

##### 4.1 *Cronobacter sakazakii*

*C. sakazakii*, a Gram-negative pathogen associated with food-borne diseases in immunocompromised neonates. It causes symptoms like meningitis, necrotizing enterocolitis and systemic bacteremia with a mortality rate of about 33-80%. *C. sakazakii* colonized the *C. elegans* intestine and caused mortality with an  $LT_{50}$  of  $134 \pm 2.8$  h. Additionally, exposing nematodes to *C. sakazakii* for 24 h damages the intestinal tract and affects the normal gametogenesis during infection. The gene expression analysis revealed that the antimicrobial gene *lys-7* was found to be highly regulated till 72 h of infection than their respective controls. However, the genes *lec-60* and *lec-87* were appeared to be not-regulated during the course of infection [14]. *C. sakazakii* infection further altered the expression of Daf-2/Daf-16 pathway genes (*daf-2*, *daf-16*, *age-1*, *bec-1*), candidate innate immune genes and aging-related genes (*bra-2*, *clk-2*, *skn-1*, and F08G5.3). *C. sakazakii* mediated infection distorted the transcriptional regulating factor (*atf-7*), thereby it affects the controlled expression of immune players of the host (Unpublished data).

LPS is a major constituent of all Gram-negative bacteria. It acts as a potent pro-inflammatory molecule. The entry of pathogenic bacteria into the host results in the discharge of a diminutive quantity of LPS into the host system. It is the initial step of inducing the immune response of host during the infection. In response to the injected LPS, host system provokes the immune signaling pathway to neutralize further infection. This is the essential step required to protect the host, if the LPS-induced inflammatory response is uncontrolled, that will lead to the fatal sepsis [15]. The LPS isolated from *C. sakazakii* causes mortality in a dose-dependent manner as the consequences of inhibiting the reproduction and feeding in nematodes. The impact of LPS intervention on major immune regulatory and aging-related pathway genes has been studied. The endotoxin LPS alone affects the selected host defensive (*lys-7*, *ilys-3*, *lec-60*, *lec-87*, F08G5.6,

*atf-7*, *scl-2*, *cpr-2*) and aging-related genes (*skn-1*, *clk-2*, *bra-2*, *age-1*, *daf-16*, *daf-2*, *bec-1*). Also, the conserved p38 MAPK pathway appear to play a key role in *C. elegans* immune response against *C. sakazakii* LPS-mediated challenges. On *C. sakazakii* front, the LPS of the pathogen was structurally modified by stretching and bending its –CN– and N–H groups during interaction with host. Additionally, the mixed region of the LPS, which coded vibration for phosphate-carrying compounds, peptides and fatty acid region also varied upon interaction with the host when compared to naive bacteria [16]. These modifications were suggested to be contributed for the escape of the *C. sakazakii* from host defensive action.

#### 4.2 *Klebsiella pneumoniae*

*K. pneumoniae* is a Gram-negative bacterium ranked second in causing both nosocomial- and community-acquired infections. It causes a diverse array of infections such as bowel disease, pneumoniae, suppurative infections, bacteremia, septicemia and urinary tract infections. In severe cases, it causes mortality of about 20-55% by pyogenic liver abscess along with meningitis and endophthalmitis [17]. *K. pneumoniae* killed nematodes as a consequence of an accumulation and propagation of the pathogen inside the worms' intestine. The molecular analysis reveals that the infection with *K. pneumoniae* affected the reproduction by disturbing the vulval development and egg laying. This was evident by the down-regulation of *let-23*, an EGF-receptor family transmembrane tyrosine kinase responsible for vulval development and *lin-29*, a zinc finger transcription factor responsible for egg-laying [18]. In addition, the transcriptomics analysis in *C. elegans* reveals that *K. pneumoniae* attenuates the host immune system by down-regulating the p38 mitogen activated protein kinase pathway by chiefly inhibiting the production of antimicrobial factors such as *nlp-29*, *lys-1* and C-type lectins [9]. Although, infection with *K. pneumoniae* attenuates the immune system of host, it is necessary to evade the elicited immune response to cause the infection. For this reason, *K. pneumoniae* modified the C=O ester and –NH group of LPS to form a strong hydrogen interaction with the host cell (unpublished data).

Moreover, to check the hypothesis that *K. pneumoniae* utilizes same virulence strategies to infect nematode and mammalian host, gene expression analysis was performed for selective candidate virulent genes. The virulent genes including *rmpA*, *uge* and *oxyR* were well established to be the essential players for the colonization [19,20] and to withstand the oxidative stress [21]. The transcriptional analysis showed that the interaction of *K. pneumoniae* with *C. elegans* significantly increased the expression fold of virulence genes such as *rmpA*, *oxyR* and *uge* than their respective controls. The over-expression of these reported genes in *K. pneumoniae* during host-pathogen interaction suggested that the *K. pneumoniae* shares a common mode of pathogenesis between nematode and mammals (unpublished data).

#### 4.3 *Staphylococcus aureus*

*S. aureus*, a versatile Gram-positive pathogen has been capable of causing a wide range of human diseases ranging from mild illness (minor skin infections, pimples, impetigo, boils, cellulitis folliculitis, carbuncles, scalded skin syndrome and abscesses) to life-threatening infections (pneumonia, meningitis, osteomyelitis, endocarditis, bacteremia and sepsis) [22]. It produces a large number of virulence factors and extracellular toxins like alpha toxin, cell-wall associated proteins that play vital role in colonizing the host body, tissue destruction and to evade the immune system of infected host [23]. *S. aureus* required 90±10 h for the complete killing of *C. elegans*. Particularly, 20% inoculum required continuous exposure for causing mortality in nematodes, whereas, 100% inoculum required only 8 h for complete killing. This reveals that the *S. aureus* caused cell density dependent mortality in nematodes. Furthermore, the significant regulations of candidate immune regulatory genes like lysozyme (*lys-7*), cysteine protease (*cpr-2*) and C-type lectin (*clec*) family members during *S. aureus* infection decipher the possible contribution of antimicrobial players during host response against infection. During initial hours (0-12 h), there was no significant increase in the expression of *lys-7*. However, 24 h of *S. aureus* exposure showed an increased level of *lys-7* against infection. In the mean-time, the later hours of infection (36 and 48 h) suppressed the level of expression of *lys-7*. Additionally, the *cpr-2*, *clec-60* and *clec-87* found to be upregulated at 24 h and down-regulated at late hours [22]. This result clearly suggested the invading *S. aureus* infection combat the host immune defense.

Lipoteichoic acid (LTA) is one of the surface-associated molecular patterns found in Gram-positive bacteria. LTA is antigenically equivalent to lipopolysaccharide in provoking the inflammatory response against Gram-positive bacteria. The LTA isolated from *S. aureus* modified the immune system of host by altering the expression of *lys-7*, *clec-60*, *mpk-1* and *atf-7*. Gene expression analysis in *S. aureus* revealed that the gene responsible for LTA synthase and other virulence genes were found to be up regulated during host-pathogen interaction. In *S. aureus*,  $\alpha$ -toxin, a cytolytic exotoxin is very essential for the pathogenesis during the infection. Another gene, *Sak* is required for the invasion of bacteria inside the host cell. The up regulation of  $\alpha$ -toxin and *sak* during interaction with host indicated the pathogenesis of *S. aureus* during infection [24].



#### 4.4 *Shigella* spp.

*Shigellae* are the major cause for bacillary dysentery or shigellosis. *Shigella* spp. is Gram-negative intracellular pathogens that are transmitted through fecal-oral route. *Shigella* infects human by targeting the intestinal epithelial cells [25-27]. *Shigella* spp. (*S. flexneri* and *S. boydii* and *S. sonnei*) infect and cause mortality in *C. elegans* in both solid and liquid medium assays [27-29]. The semi-quantitative RT-PCR analysis of host innate immune genes revealed that the *clec-60* and *clec-87* were upregulated at the early hour of an infection and gradually down-regulated at the later hours of *S. flexneri* infection. However, the *lys-7* was found to be down-regulated at the early hours of infection and up regulated in the late hours of infection indicated the host defense against *S. flexneri* infection. The pathogen virulence gene expression analysis in *C. elegans* during *S. flexneri* pathogenesis suggested that the pathogen up regulated the virulence factors haemolysin E (*hlyE*) and flagellar filament protein (*fliC*) during initial hour of infection and could play a vital role in eliciting the immune response of host during infection [27]. The kinetic gene expression analysis of host innate immune genes (*lys-7*, *clec-60*, *clec-87*) in *C. elegans* during *S. sonnei* infection revealed its regulation and its role in host defense system upon *Shigella* infection (unpublished data). Modulation of host innate immune defense is a general mechanism facilitating the invasion of pathogens into mucosal surfaces [27]. The quantitative Real Time PCR analysis revealed that the significant upregulation of *clec-60*, *clec-87* and *lys-7* at the early hours of infection and subsequent down-regulation at the later hours of infection indicated the infection with *S. boydii* weakened the immune response of host to cause a persistent infection in *C. elegans*. Increased susceptibility of the *lys-7* mutant *C. elegans* towards *S. boydii* indicated the host requirement of *lys-7* against *Shigella* infection. The interaction of immune proteins in *C. elegans* was analyzed by protein-protein interaction (PPI) database. LYS-7 and CLEC-60 interacts with the intermediate immune proteins partners, CLEC-62, CLEC-63, F54D5.3 and ZK1320.2 and THN-2 [28]. Subsequently, the expression analysis of the interacting intermediate immune genes (*clec-61*, *clec-62*, *clec-63*, F54D5.3, W03D2.6, and ZK1320.2) revealed the role of host immune defense against *Shigella* spp. infection [28,29].

LPS is another immunostimulatory molecule found in the bacteria that cause lethal symptoms. Other than its toxic nature, LPS structure is modified by the pathogen to escape from the host immune system. The isolated LPS from *Shigella* spp. showed a dose-dependent mortality in nematodes. The toxicity of LPS is mediated by affecting the lifespan, reproduction and feeding in exposed nematodes. Exposing *C. elegans* with LPS modulates the host immune genes like *lys-7*, *clec-60*, *clec-87*, *clec-61*, *clec-62*, *clec-63*, F54D5.3, W03D2.6, ZK1320.2, *pmk-1*, *skn-1*, and *dbl-1*. On the other hand, *Shigella* spp. modifies its LPS structure to escape from the host defense system. The analysis using FT-IR revealed that *S. flexneri* and *S. sonnei* modify their amide regions. Overall, during host interaction, the *Shigella* spp. modifies the polysaccharide, mixed and fatty acid region of LPS [30].

#### 4.5 *Pseudomonas aeruginosa*

*P. aeruginosa* is a ubiquitous, versatile Gram-negative opportunistic pathogen plays a major role in causing severe illness in immune compromised patients like cystic fibrosis. This opportunistic pathogen can infect a wide range of host. The expression of virulence gene varies with the choice of host system. *P. aeruginosa* kills *C. elegans* by two ways namely fast- and slow-killing. The killing assay with the two strains of PA01 and PA14 *P. aeruginosa* revealed that PA14 was more virulent than PA01. The result also indicated that the nematode fed with PA14 survived up to 66 ± 2 h, whereas the nematodes fed with PA01 survived up to 72 ± 2 h. Additionally, the heat-killed and cell-free supernatant of *P. aeruginosa* does not affect the life span of exposed nematodes. The killing of nematodes by PA14 is a consequent of accumulation of bacteria inside the intestine of the exposed nematodes. This was evidenced by the accumulation of PA14::GFP inside the nematode intestine, whereas the strain PA01 did not colonize the intestinal layer of the nematodes'. The different strains of *P. aeruginosa* elicited different immune responses in the host. The elicitation of immune response is depending on the surface molecule of the pathogen. The immune genes *clec-87* and *lys-7* was significantly up regulated than the *clec-60* in PA14, whereas *clec-60* was not regulated significantly throughout the course of infection. The isolated LPS of PA14 and PA01 were also observed to be regulated the *clec-60*, *clec-87* and *lys-7* in a dose-dependent manner [31].

On pathogen front, *P. aeruginosa*, both PA14 and PA01 underwent structural modification by altering its LPS components polysaccharide and fatty acid to escape from the host immune defense and cause a persistent infection. PA01 modifies its pentacetylated fatty acid chain by changing the spin lattice of 2 and 2' amide group, -CH group of glucosamine found in lipid A to withstand the host immune defense [32]. Furthermore, the variation in the d-spacing and intermolecular structure of isolated LPS by X-ray diffraction analysis was evident for the structural variation in LPS of pathogens while interacting with the host. In addition to modifying its lipid A structure, *P. aeruginosa* was also reported to express a virulent gene *exoT* during infecting the nematode [31].

#### 4.6 *Vibrio* spp.

Vibrios are a Gram-negative rod shaped bacteria dwelling in marine environment and considered as serious pathogen causing sea food contamination. *V. parahaemolyticus* and *V. alginolyticus* causes acute gastroenteritis, wound infection, ear infection and septicemia in human. *Vibrio* infection occurs through the consumption of raw or partially cooked contaminated sea foods. Kanagawa phenomenon on Wagatsuma agar differentiates human pathogenic strains from the

non-pathogenic strains. Almost, all clinical isolates are considered Kanagawa positive and very few environmental isolates likely possess this property. *V. cholerae* infection on solid medium displayed complete killing of nematode in approximately 5 days and *V. vulnificus* showed TD<sub>50</sub> of the nematode in 9 days [33,34]. Studying interaction of *C. elegans* with *V. parahaemolyticus* and *V. alginolyticus* revealed the ability of these pathogens to kill the nematode in very short time period of exposure in liquid medium (24 and 48h, respectively). Infection inhibited the egg-laying ability of *C. elegans* due to immature vulval development which resulted in internal hatching leading to “bag of worms”. *Vibrio* infection displayed significant damage to the pharyngeal region blocking the food intake. Intensive colonization of *V. parahaemolyticus* and *V. alginolyticus* in *C. elegans* intestine was confirmed using bacteria tagged with fluorescent protein (GFP). Infection with *Vibrio* spp. upregulated the expression of immune responsible genes (*lys-7*, *clec-60* and *clec-87*) during initial hours before succumbing the host and loss of *lys-7* showed increased susceptibility towards *Vibrio* infection [10,11]. Investigation of *C. elegans* proteome against *V. alginolyticus* infection revealed the role of proteins involved in UPR pathway and their contribution in immune defense during infection [35].

## 5. Conclusion

Over all, *C. elegans* provide a valuable platform to understand the bacterial pathogenesis related complications and the molecular mechanism underlying it. The revelation of certain effector molecules of the innate immune system makes *C. elegans* open to future host-pathogen interaction studies.

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