ISJ 11: 228-239, 2014 ISSN 1824-307X

RESEARCH REPORT

sek-1 is important in tissue-specific regulation of innate immunity during the *Xoo* infection in the model host *Caenorhabditis elegans*

Y Bai[1], D Zhi[2], C Li[1], D Liu[1], H Ren[2], C Gao[2], X Wang[2], Y Li[2], Z Wu[2], H Li[1,2]

[1]Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution, School of Life Sciences, Lanzhou University, Lanzhou 730000, PR China
[2]School of Pharmacy, Lanzhou University, Lanzhou 730000, PR China

Accepted August 23, 2014

Abstract

Xanthomonas oryzae pv. Oryzae (Xoo) are plant pathogenic bacteria that can cause serious blight of rice. We have demonstrated that *Xoo* can infect the model organism *C. elegans* and p38 MAPK pathway plays specific roles in defense against the pathogen in our previous paper. Based on that p38 MAPK pathway can be activated in a range of tissues, it is intriguing to compare the tissue-specific activities of this pathway in host innate immunity. Here, transgenic worms that *sek-1* expressed specifically in neurons system, ciliated sensory neurons, and intestine respectively are used to determine the nematode survival and transcriptional levels of immune-related genes. We report that SEK-1 and TOL-1 are not involved in *C. elegans* avoidance behavior, and ingestion of nematodes is related to the aversion and also the characteristics of bacteria. In addition, *tol-1* and *sek-1* participate the immune response to the infection by *Xoo*; *sek-1* also exhibits tissue-specific activities in host innate immunity. Our findings suggest that overlapping immune effect may exist between the *tol-1* and *sek-1*.

Key Words: *C. elegans*; *tol-1*; *sek-1*; tissue-specific activity; innate immunity

Introduction

Caenorhabditis elegans is a bacterivore species so that microbes represent both nutrient sources and also potential infection sources (Garsin *et al.*, 2001). Diverse infection modes by pathogens have been reported in *C. elegans* (Pujol *et al.*, 2008; Irazoqui *et al.*, 2010). When infection happens, *C. elegans* appears to initiate behavioral defenses such as avoidance and reducing digestion rates (Hasshoff *et al.,* 2007; Schulenburg and Ewbank, 2007). In addition, *C. elegans* are known to regulate physiological processes including innate immunity through activating conserved neuroendocrine signal and neuropeptides (Styer *et al.*, 2008). A complex innate immune response is involved in the infection, evolutionary conserved signaling pathways, such as Toll-like receptor (TLR) signaling pathway and p38 MAPK pathway are employed to defense the pathogens invasion (Akira *et al.*, 2006). *tol-1*, a component of Toll signaling pathway, encodes a TLR, functions in pathogen recognition and thus enables *C. elegans* to avoid a potential pathogen (Pujol *et al.*, 2001). Aversion behavior indicates the favor of worm to the bacteria; it may also influence the ingestion of nematode. As the only TLR gene in *C. elegans,* however, *tol-1* appears to play no roles in innate immunity (Kanzok *et al.*, 2004). Nevertheless, TOL-1 is required for proper innate immunity in the presence of certain Gram-negative bacteria but it does not have a universal immune protection against pathogens (Tenor and Aballay, 2007).

C. elegans has no specialized immune cells like vertebrate for resisting pathogens, the antimicrobial genes are expressed in tissues where the site is exposed to the pathogen infection environment (Alper *et al.*, 2007), including the tissues that are in contact with the exterior or ingested microbes. Digestive tract (pharynx and intestine) is the primary route of infection (Alegado *et al.*, 2003; Tenor and Aballay, 2007), and is considered as the typical infection model to investigate the immune response.

Corresponding author:
Hongyu Li
Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution
School of Life Sciences
School of Pharmacy
Lanzhou University
Tianshui South Road No. 222
Lanzhou 730000, PR China
E-mail address: lihy@lzu.edu.cn

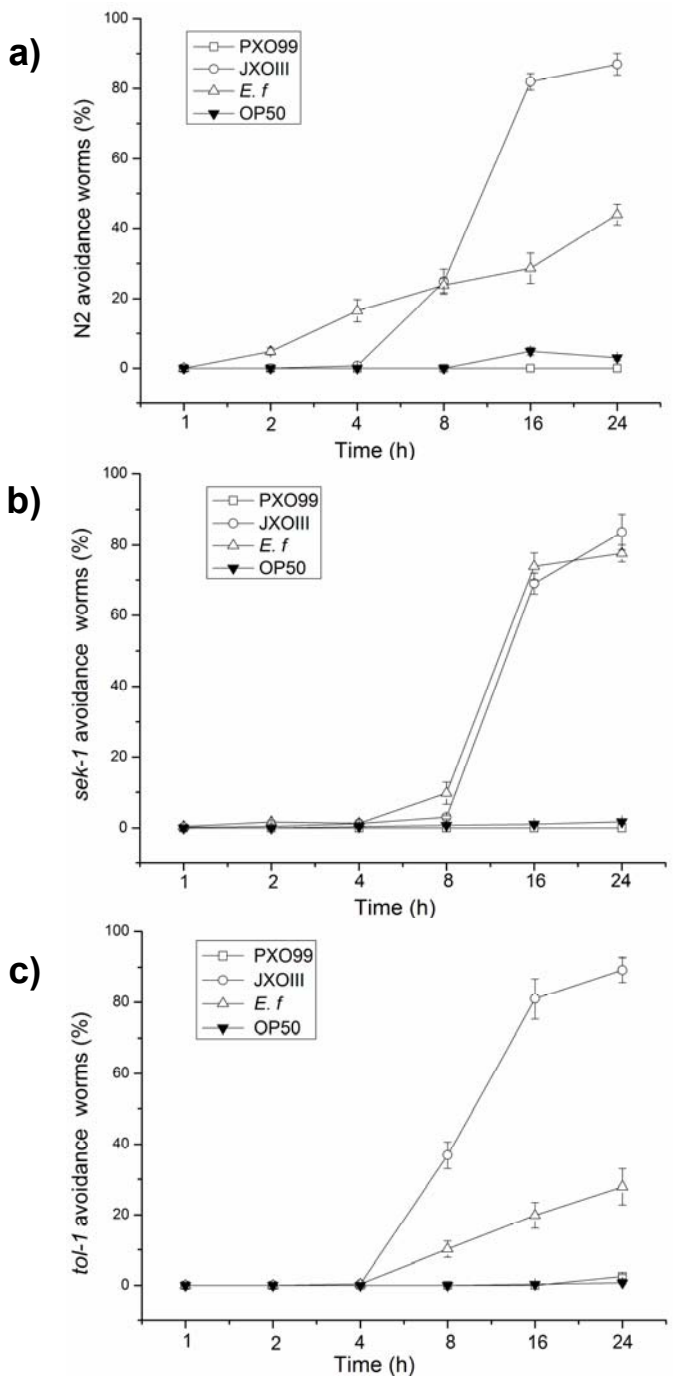

Fig. 1 *tol-1* and *sek-1* were not involved in aversion behavior. **a**) Wild type N2 worms avoided JXOIII and *E. faecalis* markedly. **b**) *sek-1* mutants exhibited similar tendency while *E. faecalis* induced stronger avoidance. **c**) *tol-1* mutation had no influence on the avoidance to the bacteria we examined.

Chemosensory system with the sensory cilia enables the worm to detect the various cues of food and danger, and then elicit chemotaxis or avoidance behavior (Bargmann, 2006). Nervous system regulates the innate immune responses to maintain the delicate balance between infection and health (Kawli *et al.*, 2010).

It is obvious that multiple immune pathways are activated upon encountering a single pathogen in *C. elegans* and the host can distinguish different pathogens and elicit specific responses (Bogaerts *et al.*, 2010). The NSY-1-SEK-1-PMK-1 p38 MAPK pathway mediates resistance to bacterial pathogens and *sek-1* mutants have enhanced susceptibility to pathogens (Kim *et al.*, 2004). p38 MAPK pathway is not involved in aversion behavior, but SEK-1

contributes to this physiological process in response to certain functional classes of gene inactivation by RNAi (Melo and Ruvkun, 2012). SEK-1 activities in different tissues exhibit specific patterns in modulating immune responses to infection. In sensory nervous system, SEK-1 is required for a protective avoidance behavior to PA14, However, SEK-1 shows a cell-autonomous regulation in intestinal immunity (Shivers *et al.*, 2009). Downstream effectors of p38 MAPK pathway like C17H12.8, F08G5.6, F56D6.2 and T24B8.5 act as immune-related genes, they are classified into CUB-like genes, C-type lectin genes and ShK toxin genes, respectively (Troemel *et al.*, 2006). Based on the higher transcriptional levels (Troemel *et al.*, 2006), we choose these genes to investigate the immune responses to pathogenic bacteria infection.

Xanthomonas oryzae pv. Oryzae (Xoo) is a plant pathogen, Gram-negative, causes serious bacterial blight of rice (Hopkins *et al.*, 1992). In our previous research, it is shown that *Xoo* can infect *C. elegans* and trigger innate immune responses to defense against the adverse effects on the host organism through up-regulating the downstream effectors. Given that immune-related components express in multiple tissues, different transgenic worms (Shivers *et al.*, 2009) are used in our research. Here, we further report that aversion behavior is benefit for nematode survival and *sek-1* is important in tissue-specific innate immunity in response to *Xoo* infection.

Materials and Methods

Bacteria and C. elegans strains

The nematode strains of wild type N2, IG10 (*tol-1(nr2033) I*), ZD193 (*sek-1(km4) X; qdEx4*) , ZD202 (*sek-1(km4) X; qdEx8*), and ZD260 (*sek-1(km4) X; qdEx11*) were provided by the *Caenorhabditis* Genetics Center (CGC). KU4 (*sek-1(km4) X*) were kindly gifted from Ausubel Lab of Department of Molecular Biology, Massachusetts General Hospital. *Xoo* strains Philippine race PXO99 and Japonic race JXOIII, were kindly supplied by Prof. JS Wang, Key Laboratory of Monitoring and Management of Plant Diseases and Pests, Ministry of Agriculture, Department of Plant Protection, Nanjing Agricultural University (Li *et al.*, 2007). *E. coli* OP50 and *Enterococcus faecalis* (ATCC29212) were used as positive and negative control, respectively. *Xoo* were maintained in nutrient agar (NA) liquid medium, *E. faecalis* was in Brain Heart Infusion (BHI) medium, and OP50 was in LB broth.

Microbial avoidance assays

Avoidance assays were generally performed as previously described (Melo and Ruvkun, 2012). Briefly, bacterial overnight cultures were concentrated 20×, and 50 ul aliquots were dropped in the centre of 3cm diameter NGM plates about 1 h before use. At least 50 synchronized L4 stage worms were dropped directly onto lawns and plates were scored for aversion ($A = N_{\text{off lawn}}/N_{\text{total}}$) at the time points of 1, 2, 4, 8, 16, 24 h. Three replica plates were prepared for each treatment and at least two independent trails were repeated.

Nematode ingestion behavior

Xoo strains were cultured at 28 °C and *E. faecalis* and *E. coli* OP50 were cultured at 37 °C until the logarithmic phase. L4 stage wild type N2 and *sek-1* mutant worms were added to 96-wells plates contained the same known concentration bacterial cultures. Each well contained 70 - 80 worms and each treatment was performed in triplicate. For 24 h and 96 h, supernatants were collected to measure the OD600 and worms were obtained through centrifuging to test the total protein concentration by Lowry method. Bacterial consumption was determined by ΔOD_{600}/protein (mg).

Body length measurement

sek-1 mutants were treated as ingestion assay described above. For 24 h and 96 h, worms were collected by centrifugation and washed twice in M9 buffer. The tube was heated to about 40 - 50 °C for several seconds to make the worms straight and easy to measure body length. Then worms were pipetted on a glass slide and covered with a coverglass. At least 30 worms were measured each group under a Motic microscope equipped with Motic Image software Advanced 3.2.

Killing assays

Killing assays were performed as described (Tan *et al.*, 1999), modified with spotting a small lawn in the centre of the plates (Shivers *et al.*, 2009). Then the plates were incubated overnight and equilibrated to the room temperature. L4 stage worms were picked to the assay plates and transferred to fresh bacterial plates every 48 h to eliminate newborn larvae. Assays for survival of ZD193, ZD202 and ZD260 were performed with big-lawn to exclude the interference of aversion. A total of 60-75 L4 larvae for each genotype were assayed in two independent trials. Worms were scored as dead and live every 24 h along the time course.

Quantitative RT-PCR

Synchronized eggs were seeded on *E. coli* OP50 plates until L4 larvae, RNA were extracted from L4 worms exposed to PXO99, JXOIII, *E. faecalis* and OP50 for 24 h using Triol (TaKaRa). cDNA was generated using the primescript RT reagent kit with gDNA Eraser (TaKaRa) as indicated by the manufacturer. The qRT-PCR was performed on a BIO-RAD S1000 Thermal Cycler using SYBR Premix Ex TaqTM Ⅱ (TaKaRa). Cycle threshold (Ct) values were normalized to *act-3*. Samples treated by pathogenic bacteria were compared to parallel samples feeding on *E. coli* OP50. Primers used were listed in Table 3.

Statistics

SPSS Ver. 17.0 was used for data processing; Kaplan-Meier method and log-rank test were adopted for statistical analysis. RT-PCR statistical tests were calculated from OP50-normalized cycle threshold values prior to conversion to relative fold change. The normalized values for induction expression for the 3 replicates were compared using a 1-sample t-test.

Results

tol-1 and sek-1 are not involved in aversion behavior

Previous work has shown that *tol-1* is required for the worm avoidance behavior of pathogenic *Serratia marcescens* (Pujol *et al.*, 2001), SEK-1 activity in the chemosensory neuron is necessary for protective avoidance response to *Pseudomonas aeruginosa* strain PA14 (Shivers *et al.*, 2009). In our study, the regular food source OP50 exerted an attractive effect on wild type N2, as well as the *Xoo* strain PXO99, that was, nearly no aversion. However, *E. faecalis* and another *Xoo* strain JXOIII had a strong tendency to repel N2 worms (Fig. 1a). Avoidance worms for 16 h was shown in Figure 2, it also indicated that part of N2 worms were out of the JXOIII and *E. faecalis* bacterial lawns (Figs 2B, C) while almost all the worms still maintained in the PXO99 and OP50 lawns (Figs 2A, D). *sek-1* and *tol-1* mutation had no effect on the avoidance tendency against JXOIII and PXO99 (Figs 1b, c), suggesting that *tol-1* and *sek-1* were not involved in aversion behavior.

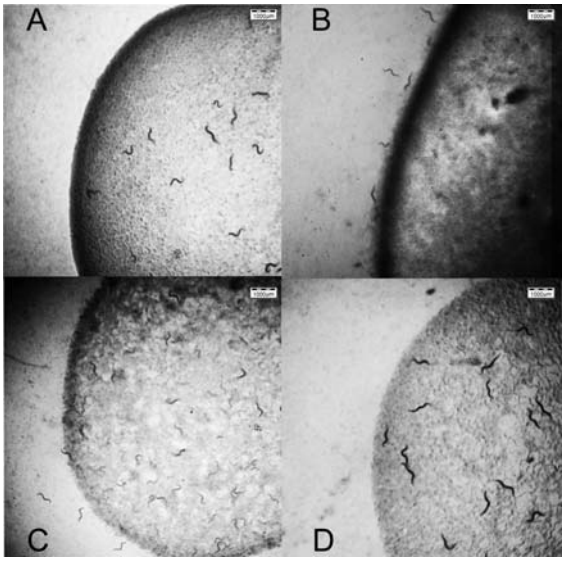

Fig. 2 Avoidance behavior of wild type N2 at the time point 16 h. Several worms were out of the bacterial lawns of JXOIII and *E. faecalis* while almost all the worms were within the PXO99 and OP50 lawns. (**A**): PXO99 (**B**): JXOIII (**C**): *E. faecalis* (**D**): OP50

Ingestion behavior is related to the body length and associate with the survival of worms

Aversion behavior is a common response to products released by pathogen (Pradel *et al.*, 2007), these substances may have an effect on ingestive efficiency of *C. elegans* (Schmeisser *et al*., 2013). Pathogenic bacterium itself also serves as cues to be detected and ingested. In our previous work, *Xoo* PXO99 and JXOIII can infect the *C. elegans* like *E. faecalis* (Bai *et al.*, 2014). To determine whether nematode ingestion is related to the favor of pathogenic bacteria, we tested bacterial consumption for different time periods. Consistent with the tendency of avoidance, ingestion of JXOIII and *E. faecalis* by both N2 and *sek-1* mutants were reduced remarkably for 24 h in contrast to their normal food source OP50 (Figs 3a, b). Bacteria that smelt or tasted unpleasantly might be avoided and not be taken up effectively (Kaletta and Hengartner, 2006). It indicated that nearly no aversion was occurred after the exposure to PXO99 like OP50 (Figs 1a, b), however, the consumption of PXO99 was significantly reduced for 24 h compared to the control (Figs 3a, b). Thus, PXO99 could escape detection by *C. elegans* but pathogenic virulence of PXO99 induced the worm reducing uptaking. It is intriguing that PXO99 and JXOIII ingestion increased as time went on and JXOIII had no significant differences compared with control group in *sek-1* mutants for 96 h (Fig. 3b). Habituation to the environment because of long-time exposure or repeated stimulation is defined as a reason for decreasing in responding (Rose and Rankin, 2001). It was hypothesized that adaption to the environment conferred the worm greater chances to survive and *sek-1* mutation might induce recognition defect of the worms when the exposure time is longer than 24 h. Factors affect ingestion were not only related to the characteristics of pathogen itself but also the interaction between the

Table 1 Body length of KU4 *(sek-1)* mutants treated with *Xoo* and *E. faecalis*

Treatment	24 h			96 h		
	Body length (mm) (means ± SD)	n	*P*-value	Body length(mm) (means ± SD)	n	*P*-value
PXO99	0.91±0.09	45	< 0.01	1.17±0.07	33	< 0.01
JXOⅢ	0.85±0.05	30	< 0.001	1.26±0.05	50	> 0.05
E. faecalis	0.73±0.07	37	< 0.001	0.92±0.11	42	< 0.001
OP50	1.04±0.12	40		1.32±0.06	37	

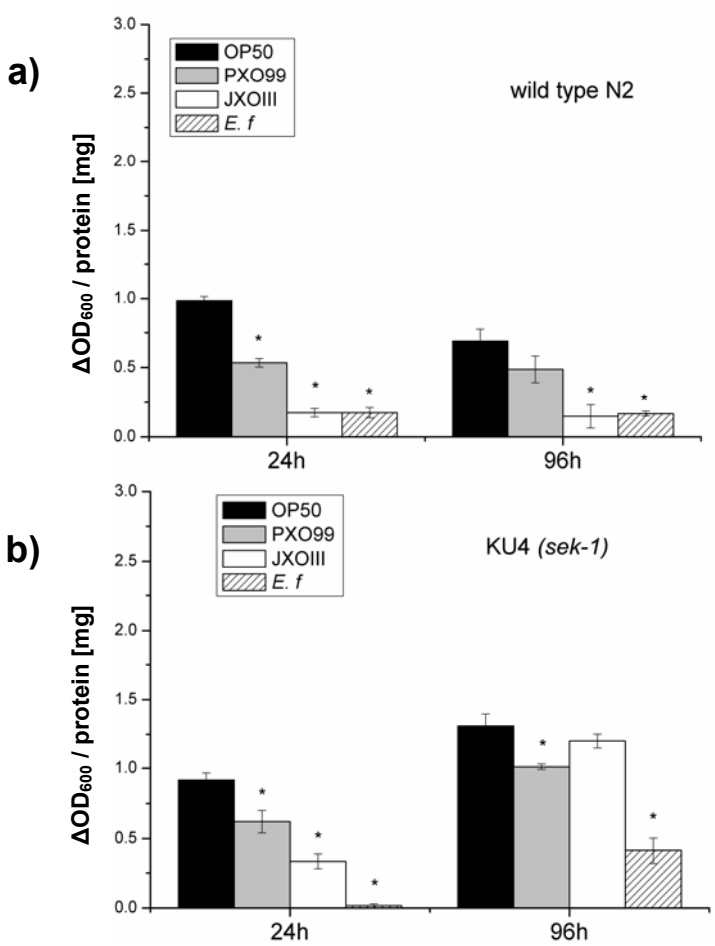

Fig. 3 Worm ingestion behaviors after exposed to pathogens and the standard food source *E. coli* OP50 for different time period. **a**) Wild type N2 ingestion changed for 24 h and 96 h. **b**) Bacteria uptaking of *sek-1* mutants for 24 h and 96 h.

host and invaders. To our knowledge, *E. faecalis* is a classic animal pathogen, colonizes and proliferates in the gut of worm (Garsin *et al*., 2001). We also reported that PXO99 and JXOIII infected *C. elegans* in a similar manner with *E. faecalis*. It is obvious that *E. faecalis* has stronger pathogenicity than *Xoo* in the worm host and animal pathogen has essential differences with phytopathogen. This might be one explanation for the more consumption of PXO99 and JXOIII in contrast to *E. faecalis*.

When compared the data in Figure 3A (24 h) to the pictures in Figure 2 (16 h), it seemed like there was a correlation between ingestion of bacteria and worm body size. Therefore, it was interesting to know if the same size difference could be seen with *sek-1* mutants. It indicated that after 96 h, all the worms we tested had longer body size than 24 h and the tendency of body length was identical to the differences of ingestion behavior (Fig. 3b, Table 1). We found that ingestion of JXOIII was fewer than PXO99 at 24 h while after 96 h the consumption of JXOIII was more than PXO99 and had no statistical significant difference with the control group. These phenomena were also verified in the body size (Table 1). It could be explained with that the PXO99 is more virulent than JXOIII (Bai *et al.,* 2014).

Behavioral avoidance promotes nematode survival to Xoo and E. faecalis exposure

Pathogenic bacteria represent an environmental challenge that can influence the worm survival, and *C. elegans* has the ability to detect and avoid pathogens (Pradel *et al.*, 2007). Avoidance behavior in small-lawn of PA14 is a natural choice response, and confers survival benefit to the nematode (Reddy *et al*., 2009). In our experiment, wild type N2 and *sek-1* mutant worms were not susceptible to both *E. faecalis* and JXOIII in small-lawn killing assay (Figs 4a, c). Compared with this, survival in the big-lawn assay in which pathogenic bacteria JXOIII were covered the entire plate performed in our previous work and other research work on identification of traditional Chinese medicine for promoting immunity of *C. elegans* against *E. faecalis* in our lab (personal communication) exhibited distinguished differences. The survival differences between small-lawn and big-lawn assays could be attributed to avoidance of the bacterial lawn (Figs 1a, b). Consistent with the

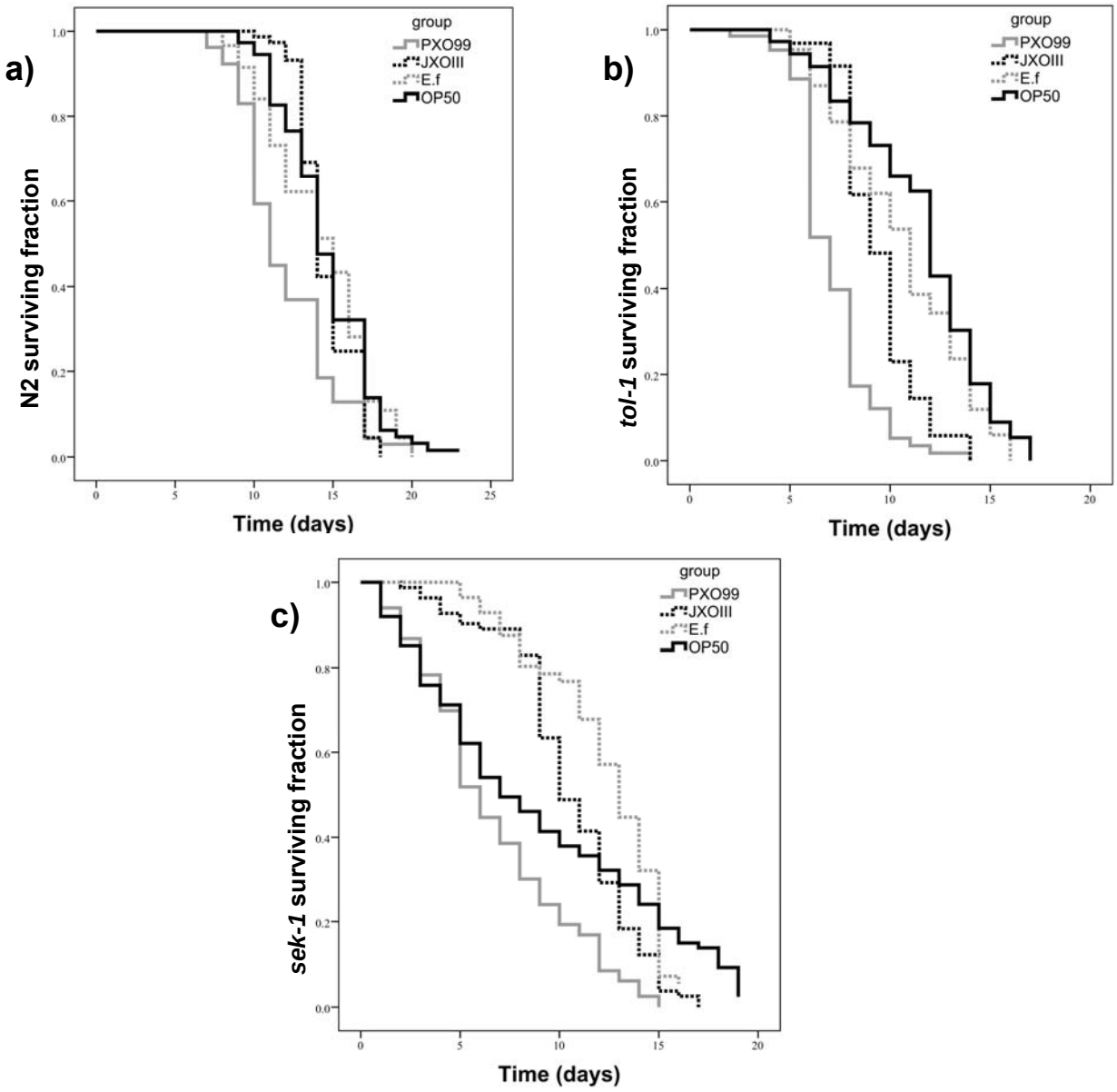

Fig. 4 Kaplan-Meier survival plots of *C. elegans* mutant strains and wild type N2 treated with *Xanthomonas* strain PXO99 and JXOIII and standard food source *E. coli* OP50. Assays were performed on small-lawn plates. PXO99 group (dark gray solid line), JXOIII group (black dotted line), *E. faecalis* group (dark gray dotted line), OP50 (black solid line).

results that *tol-1* mutants are not more susceptible to *E. faecalis* than wild-type worms (Tenor and Aballay, 2007), the *tol-1* mutants in our experimental condition also revealed similar survival patterns to N2 treated by *E. faecalis* (Fig. 4b). However, although *tol-1* mutants avoided JXOIII strongly, it seemed no benefit for survival compared to the control ($p < 0.0001$) (Fig. 4b, Table 2). This might be ascribed to the importance of TOL-1 in the innate immune response to Gram-negative bacteria (Tenor and Aballay, 2007). In contrast to *E. faecalis* and JXOIII, PXO99 decreased the survival of wild-type N2, *tol-1* mutants, and *sek-1* mutants remarkably (Figs 4a - c, Table 2). It has been proved that PXO99 is more virulent than JXOIII in our previous data (Bai *et al.,* 2014), together with the no avoidance results (Fig. 1), it indicated that PXO99 could successfully avoid the detection of immune system and shorten the lifespan of *C. elegans*.

Tissue-specific sek-1 activities regulate immune responses to Xoo and E. faecalis

sek-1 is a key component of p38 MAPK signal pathway and plays an important role in immune responses to pathogen resistance (Liberati *et al*., 2004). Despite *sek-1* was not involved in nematode

Table 2 The effect of *Xoo* and *E. faecalis* on lifespan of *C. elegans*

Strains and genotype	Treatment	LT_{50} (in days)		First and last death		p-value [a]
		Mean	SD	Min	Max	
N2, wild type	PXO99	12.0	0.4	7	20	$p < 0.0001$
	JXOⅢ	14.5	0.2	10	18	$p = 0.38$
	E. faecalis	14.2	0.4	8	20	$p = 0.72$
	OP50	14.6	0.3	9	21	
IG10, *tol-1(nr2033)*	PXO99	7.1	0.3	2	14	$p < 0.0001$
	JXOⅢ	9.4	0.4	5	14	$p < 0.0001$
	E. faecalis	10.6	0.4	5	16	$p = 0.12$
	OP50	11.5	0.5	4	17	
KU4, *sek-1(km4)*	PXO99	6.7	0.4	1	15	$p < 0.001$
	JXOⅢ	10.6	0.4	2	17	$p = 0.73$
	E. faecalis	12.2	0.5	5	16	$p = 0.11$
	OP50	8.9	0.6	1	19	
KU4, *sek-1(km4)*	PXO99	6.7	0.4	1	15	$p < 0.0001$
ZD193 (intestine)	PXO99	11.4	0.4	3	20	$p = 0.73$
ZD202 (neurons)	PXO99	9.2	0.7	5	16	$p < 0.0001$
ZD260 (ciliated sensory neurons)	PXO99	8.0	0.6	5	14	$p < 0.0001$
N2, wild type	PXO99	12.0	0.4	7	20	

[a] *p*-value (log rank test) compared with control group of OP50.

avoidance behavior to *Xoo* and *E. faecalis*, we proposed that tissue-specific *sek-1* expressions participate in innate immunity of *C. elegans*. Based on that no aversion was occurred when wild type N2 and *sek-1* mutants exposed to PXO99, and both small-lawn and big-lawn could supply enough bacterial sources, we chose N2 and *sek-1* mutants survival curves from experiments on 'small lawn' and ZD260, ZD202 and ZD193 survival curves from 'big-lawn' assay for comparison. *sek-1* mutation caused increased susceptibility to PXO99 ($p < 0.0001$) while transgenosis compromise the damage in different degrees (Fig. 5). Consistent with previous study that intestine is the main position where infection and immune response occur (Garsin *et al*., 2001), intestinal activity of *sek-1* (ZD193 strain) was sufficient for nematode innate immune responses to PXO99 (Fig. 5, Table 2), survival curve had no significant difference from N2. ZD260 and ZD202 strains, *sek-1* expressed in ciliated sensory neurons and all neurons respectively, both were susceptible to PXO99 like *sek-1* mutants (Fig. 5, Table 2). However, compared with *sek-1* mutants, ZD202 (*sek-1* in neuron cells) could alleviate the damage of pathogen PXO99 ($p < 0.01$, Fig. 5), ZD260 (*sek-1* in ciliated neurons) had no difference on survival ($p = 0.23$, Fig. 5). It suggested that *sek-1* expressed in neuron cells conferred the host capability to resist pathogen infection. Consistent with the survival assay, transcriptional levels of the CUB-like genes C17H12.8 and F08G5.6 were higher in ZD202 than in ZD260 (Figs 6a, b).

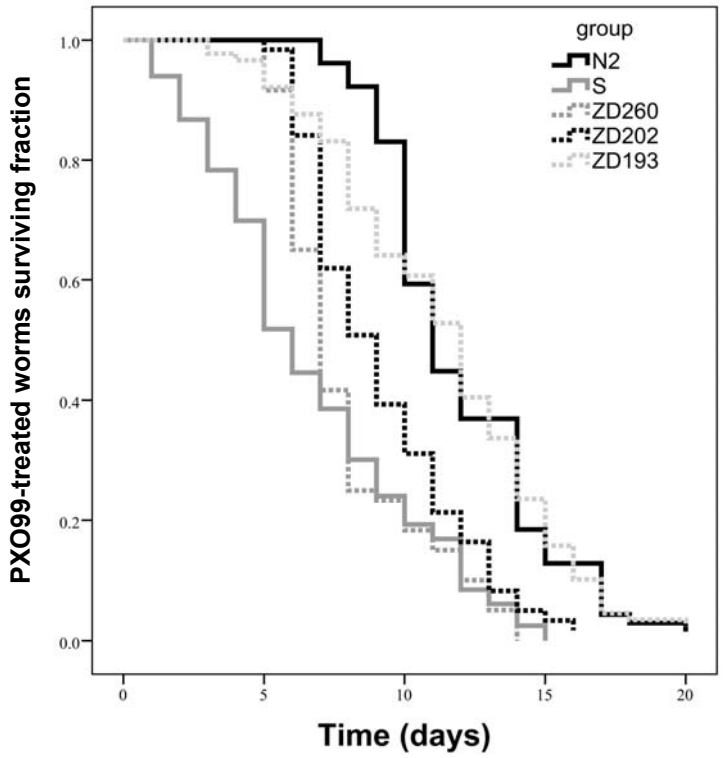

Fig. 5 Survival plots of worms treated with *Xanthomonas* strain PXO99. Small-lawn assay results of wild type N2 and *sek-1* mutants treated by PXO99 were chosen for control groups, which owing to that N2 and *sek-1* mutants showed no avoidance towards PXO99 and enough bacterial resources could be supplied on small-lawn plates like big-lawn. Big-lawn method was employed for transgenic nematodes ZD193, ZD202 and ZD260, which *sek-1* expressed specifically in intestine, neurons system, and ciliated sensory neurons. PXO99-treated wild type N2 (black solid line), *sek-1* mutant (dark gray solid line), ZD260 (dark gray dotted line), ZD202 (black dotted line), ZD193 (light gray dotted line).

Downstream genes C17H12.8, F08G5.6, T24B8.5, and F56D6.2, act as immune effectors and expression levels are enhanced significantly to defend infection attack (Troemel *et al.*, 2006). CUB-like genes C17H12.8 and F08G5.6 were significantly upregulated in wild type N2 (Figs 6a, b), together with the upregulated C-type lectin gene F56D6.2 (Fig. 6c), it showed that PXO99, JXOIII and *E. faecalis* could activate p38 MAPK pathway to defend against infection. s*ek-1* mutation led to no response that all these effector genes nearly had no transcript (Figs 6a - d). However, tissue-specific expression of *sek-1* could rescue the defense response. ZD193, *sek-1* specifically expressed in the intestine, effector genes C17H12.8, F08G5.6, F56D6.2 and T24B8.5 were remarkably upregulated after treated by PXO99 and JXOIII (Figs 6a - d). Among these results, JXOIII could induce higher gene transcriptional levels (Figs 6a, b and d), it further verified that JXOIII was less virulent than PXO99. ZD202 (*sek-1* in neuron cells) and ZD260 (*sek-1* in ciliated neurons) could trigger lower gene expressions compared to ZD193 (*sek-1* in intestine) (Figs 6a - d).

tol-1 did not participate the avoidance response (Figs 1a, c) but it may be involved in immune response to Gram-negative bacteria like *Xoo* (Fig. 4b). During the infection process, C17H12.8 expression level in *tol-1* mutants increased significantly after treated by PXO99 and JXOIII than control OP50, but obviously reduced compared to the wild type N2 (Fig. 6a). Other effector genes we investigated did not have fold changes (Figs 6b - d).

Discussion

As a bacterivore species, *C. elegans* encounters various bacteria including food source and dangerous pathogens in its habitat. Avoidance behavior and conserved innate immune response are usually employed to defense against pathogenic bacteria (Reddy *et al.*, 2009). *E. faecalis* and JXOIII are animal and plant pathogens respectively, it indicated that worms could avoid the two strains effectively in the small-lawn assay (Fig. 1). Avoidance may related to the characteristics of the molecules produced by bacteria, such as the cyclic lipodepsipentapeptide serrawettin W2 produced by *Serratia* (Pradel *et al.*, 2007), and molecules including toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxin C (SEC) secreted by *Staphylococcus aureus* (Osanai *et al*., 2012). It is necessary to elaborate the mechanism of aversion by *C. elegans*, however, based on the fact that the

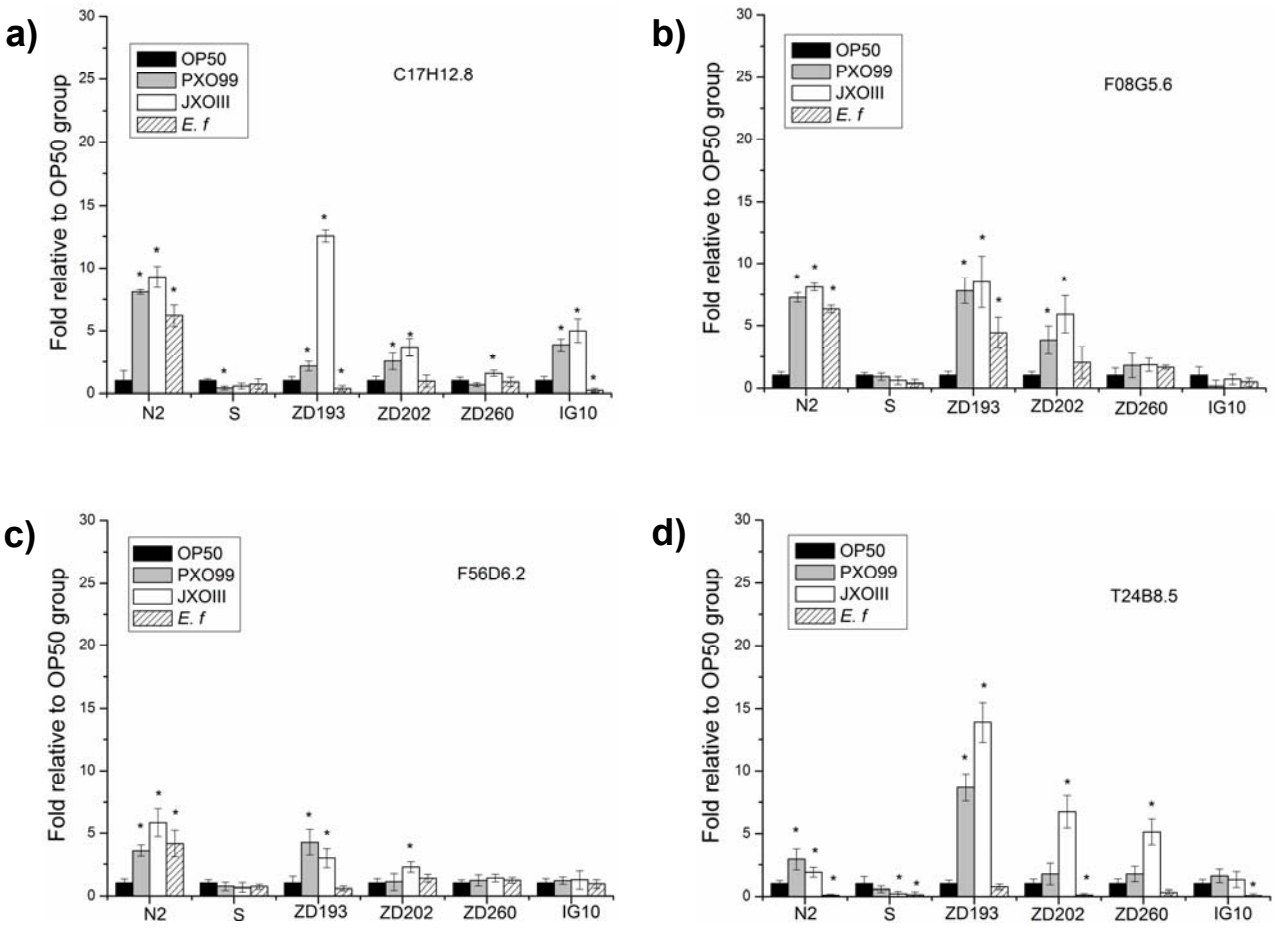

Fig. 6 Transcriptional levels of effector molecules downstream of p38 MAPK pathway after exposed to pathogenic bacteria. CUB-like genes C17H12.8 **a**) and F08G5.6 **b**), C-type lectin gene F56D6.2 **c**), and ShK toxin gene T24B8.5 **d**). We examined the fold changes of these genes to testify the roles of *tol-1* and tissue-specific *sek-1* in worm innate immunity to defend against the *Xoo* and *E. faecalis*. Results were obtained from 2 independent biological replicate and normalized to the control *act-3*. Error bars represent SD. *t-test, $p < 0.05$ comparison to OP50 groups.

issue we want to discuss is the *tol-1 and sek-1* roles in aversion and innate immunity of worms, molecules induced avoidance response are not included in our study.

Compared to the JXOIII, another *Xoo* strain PXO99 did not induce worm avoidance response. We speculate that because PXO99 is more virulent than JXOIII, it may escape the detection and recognition system of the worm and make the worm maintain in the bacterial lawn to induce greater damage. *sek-1* locates downstream of *nsy-1*, which are components of p38 MAPK pathway in *C. elegans* innate immune system (Kim *et al.*, 2002). *sek-1* mutation led to more sensitive to *E. faecalis* while there was no change in response to *Xoo* (Fig. 1b). Consistent with the importance of *nsy-1* mutation in enhanced avoidance (Mori, 2008), *sek-1* mutation may also cause more susceptibility to *E. faecalis* (Fig. 1b). *tol-1* is important for nematode in pathogen recognition, and that *tol-1* mutants are defective in their aversion of pathogenic *Serratia marcescens* (Pujol *et al.*, 2001). However, *tol-1* mutation seemed have no forward or reversal effect on the aversion in our study (Fig. 1C); it suggested that *tol-1* did not play a universal role in recognizing pathogens and triggering avoidance response. In addition, *sek-1* was not involved in aversion to pathogenic *Xoo*.

Aversion is an indicator of unpleasant odorant or taste sensed by *C. elegans*. In order to study whether avoidance can influence the ingestion behavior, we examined bacterial consumption with wild type N2 and *sek-1* mutants for 24 h and 96 h. As regular food source, OP50 consumption was the most (Fig. 3). No aversion was exhibited when exposed to PXO99 (Fig. 1), thus uptaking of this bacterium seemed more than JXOIII and *E. faecalis* in N2 (Fig. 3A). However, when prolonging the exposure time until 96 h, consumption of bacteria all increased (Fig. 3B). We propose two explanations for this issue, (i) adaption to odorant or chemical repellents may attenuate responses to these stimuli (Colbert and Bargmann, 1995; Hilliard *et al*., 2005), so that ingestion of bacteria increased. (ii) *sek-1* was

Table 3 Sequence information for primers used in qRT-PCR

Gene	sequence name	primer sequence
C17H12.8	C17H12.8	5'TGTCATTTCAATGGAGGATATTGT3'
		5'TGATGGAGTTGGAGGATATTGA3'
F08G5.6	F08G5.6	5'CACAATGATTTCAATGCGAGA3'
		5' TGCTTTCAGAACACAGTCAGG3'
F56D6.2	F56D6.2	5' TGATGGTGACAGTTCAAAGC3'
		5' TTCCAAAAATGCCCGAGTAG3'
T24B8.5	T24B8.5	5' AGACCATCATGCCCTTCACT3'
		5' GTAACGCAGACACCACAGGT3'
act-3	T04C12.4	5'CCATCATGAAGTGCGACATTG3'
		5'CATGGTTGATGGGGCAAGAG3'

not involved in avoidance behavior to *Xoo* (Figs 1a, b), nevertheless, it plays important roles in *C. elelgans* innate immunity to resist bacterial pathogens (Kim *et al.*, 2004). Mutation in *sek-1* impairs the defense capability of the host, and pathogens can escape the detection system of worms and enter into the digestive tract. In addition, virulence of pathogens is also a key factor to influence the ingestion behavior. In our previous study, *E. faecalis* is more virulent to nematode than JXOIII (Bai *et al*., 2014), it may enter the host digestive tract easier than JXOIII as we expected. However, although JXOIII and *E. faecalis* could be both avoided by *sek-1* mutants effectively (Fig. 1b), JXOIII could be ingested more than *E. faecalis* (Fig. 3b). This may due to differences in virulent factors such as extracellular enzymes or extracellular polysaccharides used by these pathogens (Shen and Ronald, 2002).

Lifespan assay is always used to evaluate pathogenic bacterial effect on *C. elelgans* and defense responses of various mutant hosts to pathogens (Irazoqui *et al*., 2010). In order to testify the *tol-1* and *sek-1* roles in host innate immunity, we performed small-lawn killing assay using *tol-1* and *sek-1* mutants. Based on the aversion of JXOIII and *E. faecalis* (Fig. 1b), survival of *sek-1* mutants treated by the two strains had no differences compared to the control (Fig. 4c, Table 2). However, similar aversion tendency did not induce semblable survival results in *tol-1* mutants (Figs 1c, 4b), nematodes treated with JXOIII exhibited marked lifespan differences compared with control OP50 (Fig. 4b, Table 2). It indicated that *tol-1* may participate the innate immune response to Gram-negative bacterium *Xoo* (Tenor and Aballay, 2007). Although *tol-1* and *sek-1* are both important in host immune response to *Xoo*, *tol-1* is considered to be independent of p38 MAPK pathway (Aballay *et al.*, 2003). However, transcriptional level of CUB-like gene C17H12.8 increased when *tol-1* mutants treated by *Xoo* PXO99 and JXOIII, and other genes we examined nearly had no changes (Fig. 6). We hypothesize that *tol-1* and *sek-1* may have overlapping roles in defense against the *Xoo*, and the host integrates multiple cues to respond the environmental stimulus. Interaction between *tol-1* and *sek-1* needs further investigation.

p38 MAPK pathway acts as a pivotal immune signaling module in *C. elegans*, it can be activated in a range of tissues where specific immune responses are triggered (Bolz *et al.*, 2010). Intestinal epithelium and nervous system are the common focus for investigating immune defense mechanism (Pukkila-Worley and Ausubel, 2012; Takeda and Ichijo, 2002). Therefore, transgenic worms expressed *sek-1* in ciliated sensory neurons (ZD260), neurons system (ZD202) and intestine (ZD193) were used to study the roles of tissue-specific *sek-1* in innate immune response to *Xoo* infection. It showed that intestine expression could rescue the survival of worms while neurons system and ciliated sensory neurons expression just attenuated the reduced lifespan compared to *sek-1* mutants (Fig. 5, Table 2). Consistent with this, effector molecules downstream of p38 MAPK pathway such as C17H12.8, F08G5.6, F56D6.2, and T24B8.5 had enhanced transcriptional levels in ZD193 (intestine expression) (Fig. 6). Fold changes of C17H12.8 and F08G5.6 were higher in ZD202 (neurons) than ZD260 (ciliated sensory

neurons) (Fig. 6), it suggested that *sek-1* expressed in neurons could confer the host limited resistance to the *Xoo*.

In conclusion, our findings reveal that SEK-1 and TOL-1 are not involved in *C. elegans* avoidance behavior and ingestion of nematodes is related to the aversion and also the characteristics of bacteria. In addition, *tol-1* and *sek-1* participate the immune response to the infection by *Xoo*, *sek-1* also exhibits tissue-specific activities in host innate immunity. Overlapping effect may exist between the *tol-1* and *sek-1*.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31071335); the Special International Cooperation Project of MOST (2012DFA30480); the Key Project of Gansu Province Science and Technology (1002WKDE55); Gansu Province International Science and Technology Cooperation Project (090WCGA900); the Fundamental Research Funds for the Central Universities of China (lzujbky-2013-71, lzujbky-2013-75, lzujbky-2014-147, lzujbky-2014-149, and lzujbky-2014-150). The nematode strains used in our work were provided by *Caenorhabditis* Genetics Center (CGC).

References

Aballay A, Drenkard E, Hilbun LR, Ausubel FM. *Caenorhabditis elegans* innate immune response triggered by *Salmonella enterica* requires intact LPS and is mediated by a MAPK signaling pathway. Curr. Biol. 13: 47-52, 2003.

Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 124: 783-801, 2006.

Alegado RA, Campbell MC, Chen WC, Slutz SS, Tan MW. Characterization of mediators of microbial virulence and innate immunity using the *Caenorhabditis elegans* host-pathogen model. Cell. Microbiol. 5: 435-444, 2003.

Alper S, McBride SJ, Lackford B, Freedman JH, Schwartz DA. Specificity and complexity of the *Caenorhabditis elegans* innate immune response. Mol. Cell. Biol. 27: 5544-5553, 2007.

Bai YL, Zhi DJ, Li ChH, Liu DL, Zhang J, Tian J, *et al*. Infection and immune response in the nematode *Caenorhabditis elelgans* elicited by phytopathogen *Xanthomonas*. J. Microbiol. Biotechn. 2014 [im press].

Bargmann CI. Chemosensation in *C. elegans*. WormBook, ed. The *C. elegans* Research Community, doi/10.1895/ wormbook.1.123.1, http://www.wormbook.org. pp.1-29, 2006.

Bogaerts A, Beets I, Schoofs L, Verleyen P. Antimicrobial peptides in *Caenorhabditis elegans*. Inv. Surv. J. 7: 45-52, 2010.

Bolz DD, Tenor JL, Aballay A. 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, 2010.

Colbert HA, Bargmann CI. Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. Neuron 14: 803-812, 1995.

Garsin DA, Sifri CD, Mylonakis E, Qin X, Singh KV, Murray BE, *et al*. A simple model host for identifying Gram-positive virulence factors. Proc. Natl. Acad. Sci. 98: 10892-10897, 2001.

Hasshoff M, Böhnisch C, Tonn D, Hasert B, Schulenburg H. The role of *Caenorhabditis elegans* insulin-like signaling in the behavioral avoidance of pathogenic *Bacillus thuringiensis*. FASEB J. 21: 1801-1812, 2007.

Hilliard MA, Apicella AJ, Kerr R, Suzuki H, Bazzicalupo P, Schafer WR. In vivo imaging of *C. elegans* ASH neurons: cellular response and adaptation to chemical repellents. EMBO J. 24: 63-72, 2005.

Hopkins C, White F, Choi S, Guo A, Leach J. Identification of a family of avirulence genes from *Xanthomonas oryzae pv. oryzae*. Mol. Plant Microbe In. 5: 451-459, 1992.

Irazoqui JE, Troemel ER, Feinbaum RL, Luhachack LG, Cezairliyan BO, Ausubel FM. Distinct pathogenesis and host responses during infection of *C. elegans* by *P. aeruginosa* and *S. aureus*. PLoS Pathog. 6: e1000982, 2010.

Kaletta T, Hengartner MO. Finding function in novel targets: *C. elegans* as a model organism. Nat. Rev. Drug Discov. 5: 387-399, 2006.

Kanzok SM, Hoa NT, Bonizzoni M, Luna C, Huang Y, Malacrida AR, *et al*. Origin of Toll-like receptor-mediated innate immunity. J. Mol. Evol. 58: 442-448, 2004.

Kawli T, He F, Tan MW. It takes nerves to fight infections: insights on neuro-immune interactions from *C. elegans*. Dis. Mod. Mech. 3: 721-731, 2010.

Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, Inoue H, *et al*. A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. Science 297: 623-626, 2002.

Kim DH, Liberati NT, Mizuno T, Inoue H, Hisamoto N, Matsumoto K, *et al*. Integration of *Caenorhabditis elegans* MAPK pathways mediating immunity and stress resistance by MEK-1 MAPK kinase and VHP-1 MAPK phosphatase. Proc. Natl. Acad. Sci. 101: 10990-10994, 2004.

Li X, Li H, Pang X, Feng H, Zhi D, Wen J, *et al*. Localization changes of endogenous hydrogen peroxide during cell division cycle of *Xanthomonas*. Mol. Cell. Biochem. 300: 207-213, 2007.

Liberati NT, Fitzgerald KA, Kim DH, Feinbaum R, Golenbock DT, Ausubel FM. Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. Proc. Natl. Acad. Sci. 101: 6593-6598, 2004.

Melo JA, Ruvkun G. Inactivation of conserved *C. elegans* genes engages pathogen-and xenobiotic-associated defenses. Cell 149: 452-466, 2012.

Mori I. A single sensory neuron directs both attractive and repulsive odor preferences. Neuron 59: 839-840, 2008.

Osanai A, Hu DL, Nakane A. *Caenorhabditis elegans* avoids *staphyloc*occal superantigenic toxins via 5-hydroxytryptamine-dependent pathway. Can. J. Microbiol. 58: 1268-1277, 2012.

Pradel E, Zhang Y, Pujol N, Matsuyama T, Bargmann CI, Ewbank JJ. Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 104: 2295-2300, 2007.

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

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan MW, *et al*. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. Curr. Biol. 11: 809-821, 2001.

Pukkila-Worley R, Ausubel FM. Immune defense mechanisms in the *Caenorhabditis elegans* intestinal epithelium. Curr. Opin. Immunol. 24: 3-9, 2012.

Reddy KC, Andersen EC, Kruglyak L, Kim DH. A polymorphism in *npr-1* is a behavioral determinant of pathogen susceptibility in *C. elegans*. Science 323: 382-384, 2009.

Rose JK, Rankin CH. Analyses of habituation in *Caenorhabditis elegans*. Learn. Memory 8: 63-69, 2001.

Schmeisser S, Schmeisser K, Weimer S, Groth M, Priebe S, Fazius E, *et al*. Mitochondrial hormesis links low-dose arsenite exposure to lifespan extension. Aging cell 12: 508-517, 2013.

Schulenburg H, Ewbank JJ. The genetics of pathogen avoidance in *Caenorhabditis elegans*. Mol. Microbiol. 66: 563-570, 2007.

Shen Y, Ronald P. Molecular determinants of disease and resistance in interactions of *Xanthomonas oryzae pv. oryzae* and rice. Microbes and infection 4: 1361-1367, 2002.

Shivers RP, Kooistra T, Chu SW, Pagano DJ, Kim DH. Tissue-Specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in *C. elegans*. Cell Host Microbe 6: 321-330, 2009.

Styer KL, Singh V, Macosko E, Steele SE, Bargmann CI, Aballay A. Innate immunity in *Caenorhabditis elegans* is regulated by neurons expressing NPR-1/GPCR. Science 322: 460-464, 2008.

Takeda K, Ichijo H. Neuronal p38 MAPK signalling: an emerging regulator of cell fate and function in the nervous system. Genes Cells 7: 1099-1111, 2002.

Tan MW, Mahajan-Miklos S, Ausubel FM. Killing of *Caenorhabditis elegans* by *Pseudomonas aeruginosa* used to model mammalian bacterial pathogenesis. Proc. Natl. Acad. Sci. 96: 715-720, 1999.

Tenor JL, Aballay A. A conserved Toll-like receptor is required for *Caenorhabditis elegans* innate immunity. EMBO Rep. 9: 103-109, 2007.

Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH. p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. PLoS Genet. 2: e183, 2006.