

Phycocerythrin extends life span and health span of *Caenorhabditis elegans*

Ravi Raghav Sonani · Niraj Kumar Singh ·
Anjali Awasthi · Birendra Prasad · Jitendra Kumar ·
Datta Madamwar

Received: 4 June 2014 / Accepted: 26 September 2014 / Published online: 11 October 2014
© American Aging Association 2014

Abstract In the present study, we tested the antioxidant activity of phycocerythrin (PE, an oligomeric light harvesting protein isolated from *Lyngbya* sp. A09DM) to curtail aging effects in *Caenorhabditis elegans*. Purified PE (100 µg/ml) dietary supplement was given to *C. elegans* and investigated for its anti-aging potential. PE treatment improved the mean life span of wild type (N2)-animals from 15±0.1 to 19.9±0.3 days. PE treatment also moderated the decline in aging-associated physiological functions like pharyngeal pumping and locomotion with increasing age of N2 worms. Moreover, PE treatment also enhanced the stress tolerance in 5-day-aged adults with increase in mean survival rate from 22.2±2.5 to 41.6±2.5 % under thermo stress

and from 30.1±3.2 to 63.1±6.4 % under oxidative (hydrogen peroxide)-stress. PE treatment was also noted to moderate the heat-induced expression of human amyloid-beta(Aβ₁₋₄₂) peptide and associated paralysis in the muscle tissues of transgenic *C. elegans* CL4176 (Alzheimer's disease model). Effectiveness of PE in expanding the life span of mutant *C. elegans*, knockout for some up (*daf-2* and *age-1*)- and down (*daf-16*)-stream regulators of insulin/IGF-1 signaling (IIS), shows the independency of PE effect from DAF-2–AGE-1–DAF-16 signaling pathway. Moreover, the inability of PE in expanding the life span of *hsf-1* knockout *C. elegans*(*sy441*) suggests the dependency of PE effect on heat shock transcription factor (HSF-1) controlling

Ravi Raghav Sonani and Niraj Kumar Singh contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s11357-014-9717-1) contains supplementary material, which is available to authorized users.

R. R. Sonani · D. Madamwar (✉)
BRD School of Biosciences, Sardar Patel University,
Vadtal Road, Satellite Campus, Post Box No- 39, Vallabh
Vidyanagar, Gujarat 388 120, India
e-mail: datta_madamwar@yahoo.com

N. K. Singh
Department of Biotechnology, Shri A. N. Patel PG Institute,
Anand, Gujarat 388 001, India

A. Awasthi
Department of Biological Sciences, Birla Institute of
Technology and Science,
Pilani, Rajasthan 333 031, India

B. Prasad · J. Kumar
Department of Botany/Biotechnology, Patna University,
Patna, Bihar 800005, India

J. Kumar (✉)
The Buck Institute for Research on Aging,
8001 Redwood Boulevard, Novato, CA 94945, USA
e-mail: jitendra343@gmail.com

stress-induced gene expression. In conclusion, our results demonstrated a novel anti-aging activity of PE which conferred increased resistance to cellular stress resulting in improved life span and health span of *C. elegans*.

Keywords Phycoerythrin · *Caenorhabditis elegans* · Anti-aging · Life span · Health span · *Lyngbya* sp. A09DM

Introduction

Marine algae are a rich source of biologically active compound, some of which includes carotenoids, fatty acids, polysaccharides, proteins, and peptides. Protein and peptides present in these marine algae are reported to have various biomedical applications (Lordan et al. 2011) due to presence of plenty of essential amino acids in their constitution (Taboada et al. 2010). Phycobiliproteins (PBPs) constitute around 60 % of the total protein content and 20 % of the dry cell weight of cyanobacteria (Soni et al. 2008). Till date, they have been reported as potent pharmacological and medicinal agent having hepato-protective (Vadiraja et al. 1998), anti-inflammatory (Romay et al. 2003), anti-cancerous (Zhang et al. 2011), anti-Alzheimer's disease (Singh et al. 2014), and antioxidant activity (Sonani et al. 2014; Soni et al. 2008). Moreover, PBPs are also utilized as natural colorants in food products like chewing gums, dairy products, jellies as well as in cosmetics (Rasmussen and Morrissey 2007). Phycoerythrin (PE), a majorly produced PBP, is an oligomeric pigment protein, consisting of two chromophore/s-appended dissimilar polypeptide chain belonging to two different families (α and β) (Parmar et al. 2011). PE, isolated from marine cyanobacterium *Lyngbya* sp. A09DM, has shown strong and wide range of antioxidant activity in vitro against various reactive oxygen species (Sonani et al. 2014). Moreover, toxicity analysis of purified PE has approved that it is nontoxic and safe for oral consumption (unpublished data).

“Aging” is believed to be the manifestation of two major phenomena, the accumulation of reactive oxygen species (ROS) and downstream effects of insulin/insulin-like growth factor signaling (IIS) pathway (Blagosklonny 2008). There are many theories proposed for aging, out of which, free radical theory is the most prevalent. It suggests that an accumulation of free radicals and damage cause by them is the major cause of

aging (Harman 1998). Several lines of evidences proposed that increase in endogenous antioxidant production or supplement of exogenous antioxidant moderates aging by averting the free radicals (Melov et al. 2000). This influential theory offered doorway to investigate anti-aging effects of various antioxidant compounds using different model organisms (Gems and Doonan 2009). Several studies have reported beneficial effects of exogenous antioxidant supplements like vitamin E (α -tocopherol) (Harrington and Harley 1988), trolox (Ishii et al. 2004), and α -lipoic acid (Benedetti et al. 2008; Brown et al. 2006) on the manifestation of aging. Despite of having such strong lines of evidences, oxidative damage is not accepted as a sole cause for aging due to inefficacy of some antioxidants in averting aging effects (Bjelakovic et al. 2007; Schulz et al. 2007). Reduction in function of any components of the IIS pathway leads to the activation of a series of genes involved in life span control, stress tolerance, and protein misfolding suppression (Ogg et al. 1997; Lin et al. 1997), collectively resulting in life span extension (Kenyon 2010). Finding pharmacological compounds having inhibitory role in the IIS pathway has been a major research motivation for many research groups.

The aging process in *C. elegans* is quite conserved and portrays similarity to humans in some aspects like decreased motility, decreased cognitive abilities, immunosenescence, decreased rate of reproduction, and increased accumulation of harmful metabolites like lipofuscin (Iwasa et al. 2010). *C. elegans* is thus considered as a good model to study aging and testing the therapeutic compounds which may curtail aging. Use of PE as a nutraceutical food supplement for its anti-oxidative, anti-inflammatory, immunomodulatory, and antihyperalgesic properties has been reported (Soni et al. 2008; Cian et al. 2012). However, the effect of antioxidant properties of PE on aging is not explored. In this paper, we explored the potential of PE as an anti-aging drug. The effect of PE on life span and signs of aging was tested using wild type (N2 Bristol) and various mutant *C. elegans* strain. Our results demonstrate that PE treatment enhanced the life span of *C. elegans* and also decreased the rate of progression of aging-related surrogate physiological markers like decline in the rate of pharyngeal pumping and locomotion. PE treatment also conferred better tolerance to thermal and oxidative stress. As various defensive mechanisms of *C. elegans* against aging have been hypothesized to occur either via downregulation of DAF-2–AGE-1–

DAF-16 insulin signaling pathway (Kenyon 2010; Narasimhan et al. 2009; Samuelson et al. 2007; Murphy and Hu 2013; Cai et al. 2011) or via scavenging of reactive oxygen species, we targeted to track the effect of PE with respect to these two major facets. *C. elegans* null mutants and easy knockout strategy for various controllers of the IIS pathways facilitate the rational strategy to track out the mode of action of any drug in *C. elegans*. Null mutants of *daf-2(e1370)*, *daf-16(mu86)*, and *age-1(hx546)* genes have been employed to reveal the point of action of PE across the IIS pathways of *C. elegans*. Transcription factor for heat shock (HSF-1) and oxidative stress (SKN-1) response activates expression of a series of essential proteins and molecular chaperons needed to maintain proteostasis under respective stress conditions (Morton and Lamitina 2013; Zhou et al. 2011). In *C. elegans*, expression of heat shock protein by HSF-1 has been reported to be DAF-16 dependent (Kenyon 2010). SKN-1 is a worm homolog of human nuclear factor E2-related transcription factor (Nrf) and has also been reported to be involved in the IIS pathway (Tullet et al 2008). To investigate whether the protective effect of PE is mediated through HSF-1 and SKN-1, we checked the effect of PE in wild type and CL4176 (*C. elegans* Alzheimer's disease model) animals under RNAi-mediated knockout conditions.

Thus, in the present study, we explored the potential of PE as an anti-aging drug. It systematically describes the effect of PE feeding on *C. elegans* aging. We also explored the possible pathways through which PE might be exhibiting its effects.

Materials and methods

PE preparation

Lyngbya sp. A09DM was isolated from the rocky shore of Okha, Gujarat, India. Enrichment, isolation, and identification of culture were carried out according to protocol described by Shah et al. (2001). Isolated strain was grown in artificial salt nutrient (ASN) III medium (Waterbury and Stanier 1981) under illumination (12:12 h light-dark cycles) of 36 W white fluorescent lamp which provided $130 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ flux density at the surface of the flasks. PE was obtained by resuspending cells from 30-day-old culture in the isolation buffer (20 mM Tris, pH 8.0) and further subjecting it to repetitive freeze

(−25 °C)–thaw (4 °C) cycles. The brick red supernatant, obtained after centrifugation, was purified by eluting through anion-exchange low-pressure liquid chromatography and gel permeation chromatography. Purity was affirmed by native-PAGE and UV-visible absorption spectroscopy. The resulting fractions were concentrated by ultra-filtration using a 30-kDa MWCO centrifugal device (Macrosep®, Pall Corporation). Concentrated PE was lyophilized at (−100 °C) (Sentry 2.0, VirTis, SP Scientific) and stored at 4 °C under dark condition.

C. elegans strains and cultivation

The various *C. elegans* strains used in this study were N2 Bristol (wild type), CL4176 (*dvIs27 [pAF29 (myo-3/Ab 1-42/let UTR)+pRF4 (rol-6(su1006))]*), *daf-16(mu86)*, *daf-2(e1370)*, *age-1 (hx546)*, *muIs84 pAD76 (sod-3::GFP)*, and *eat-2(ad1116)*. All of the strains were obtained from the Caenorhabditis Genetics Center (CGC) at the University of Minnesota, Minneapolis MN. All *C. elegans* strains were grown at 20 °C (unless specified) on nematode growth medium (NGM) agar plates seeded with the *E. coli* strain OP50 under standard laboratory conditions as described by Brenner (1974). The *E. coli* OP50, *E. coli* HT115(DE3), and RNAi constructs were grown in Luria-Bertani (LB) medium at 37 °C. Grown cultures were spotted (200 μl of 10^{10} cfu/ml solution) onto NGM plates and incubated at 37 °C for 24 h prior to worm inoculation. Food source including *E. coli* OP50, *E. coli* HT115(DE3), and RNAi constructs were also obtained from the CGC. The effect of PE was checked by providing the PE with NGM agar at various doses of 10, 50, and 100 $\mu\text{g ml}^{-1}$ of media.

RNAi experiments

E. coli HT115 (DE3) expressing dsRNA (of RNAi) for *hsf-1* and *skn-1* were fed to worms as described previously (Timmons and Fire 1998; Kamath and Ahringer 2003). In brief, L4-stage hermaphrodites were fed with *hsf-1* and *skn-1* (RNAi) expressing bacteria for 3 days to allow the intestinal ingestion of RNAi, and ingested worms (confirmed phenotypically) are cloned to individual wells for each gene screened. Each of these is allowed to lay eggs for 1 day. Siblings and the progeny obtained from the eggs were used as RNAi mutant/knockout for particular genes.

Worm synchronization and life span assay

To obtain age-synchronized nematodes, 5–10 gravid adults were allowed to lay egg on NGM plates for 3 h and then removed. Plates with eggs were treated with sodium hypochlorite solution (1 N NaOH+ 5 % NaOCl, Sigma-Aldrich). Treated eggs were repeatedly washed in M9 buffer (3 g of KH_2PO_4 , 6 g of Na_2HPO_4 , 5 g of NaCl, 1 ml of 1 M MgSO_4 , make up the volume to 1 L using H_2O). The eggs were further incubated in M9 and allowed to hatch overnight at 20 °C. The resulting synchronized L1-stage worms were put on standard NGM plates with *E. coli* OP50 and incubated until L4 larval stage. The group of L4-synchronized *C. elegans* were taken in fresh NGM plates for life span assay (numbers of animal (*n*) for each group is depicted in supplementary table 1). Experiment was performed in triplicates. The animals were transferred into fresh food plate every alternate day and the number of dead animals was also scored. Response against mechanical stimulus (given by prodding with a platinum pick) and pharyngeal pumping rate were taken as surrogate markers to monitor dead animals (Kenyon et al. 1993). Pharyngeal pumping rate was estimated by manually monitoring an individual animal of 5th and 10th day to count the rhythmic contractions of the pharynx over 20 s by hand counter. The mean pharyngeal pumping rate for various *C. elegans* groups (5th day—control: *n*=165, PE-treated: *n*=152; 10th day—control: *n*=110, PE-treated: *n*=145) was expressed per minute. The plot of fraction survival against time (days) was constructed by taking treatment day as first day and subjected to log-rank test to obtain the mean life span.

Stress resistance assay

For stress resistance assay, N2 worms were subjected to temperature and oxidative stress. For both assays, age-synchronized young adult worms were transferred to NGM plates with and without PE treatment. They were grown till fourth day (post adulthood) and then subjected to temperature and oxidative stress, respectively. For temperature stress, PE-treated and untreated animals were shifted from 20 to 35 °C for 12 h and the number of dead worms was counted at a regular interval of every 2 h until the 12th hour. Oxidative stress was given by washing the animals

3–4 times with M9 buffer followed by soaking them in 10 and 20 mM hydrogen peroxide (H_2O_2) solution for 2 h as described by Cai et al. (2011). Following 2 h of oxidative stress, the animals were transferred to fresh NGM plates and incubated for 16 h at 20 °C for recovery. After 16 h, live animals were scored for PE-treated and untreated condition.

Locomotion assays

Locomotion assay was performed on the 5th and 10th day adult, respectively by counting the number of body bends observed under a stereo microscope during a 30-s interval (Kumar et al. 2010). A body bend was defined as a change in the reciprocating motion of bending at the mid-body. Assay was performed on PE-treated and untreated worms.

Paralysis assays

CL4176 transgenic animals containing heat-inductive transgene of human $\text{A}\beta_{1-42}$ in muscle cells were transferred to PE-treated and untreated plates and incubated at 16 °C (permissive temperature) for 48 h followed by shifting them to 25 °C (non-permissive temperature) for 20 h, to stimulate amyloid-beta ($\text{A}\beta$) expression whose aggregation in muscle cells leads to paralysis. The number of paralyzed worms was scored using the method described by Link (2003). In brief, after 20 h of incubation at 25 °C, numbers of paralyzed animals were scored at every 2 h interval until all animals were paralyzed. Newly paralyzed worms were distinguished by looking at the “halo” of cleared bacterial lawn produced by them due to movement of only the head while feeding. Therefore, worms with halos were considered as paralyzed. Moreover, worms, not responding to mechanical stimulus, were also counted as paralyzed.

RNA isolation and real time PCR

Synchronized L1 animals of wild type were raised with and without PE at 20 °C. For RNA isolation, 1-day young adult animals were washed 2–3 times by M9 buffer followed by resuspending them in Trizol reagent (Invitrogen). Total RNA was isolated by chloroform extraction, followed by ethanol precipitation and DNase treatment. cDNA was synthesized using standard protocol. Real time PCR was carried out using PCR

master mix (Bio-Rad). HSF-1 transcripts levels were quantified by normalizing it to actin (internal control), and relative expression level of transcripts were calculated.

DAF-16::GFP and Sod-3::GFP localization assay

Synchronized population of L1 stage DAF-16::GFP and Sod-3::GFP transgenic worms were grown in PE-treated and untreated plates. Young adult worms were imaged at 10 \times using a Nikon E-800 fluorescence microscope. The filters 400/30 and 508/20 were used for excitation and emission to examine the GFP transgenic lines, respectively.

Detailed information regarding experimentation methodology and results like total number of animal used, food source used in various assays, and statistical tests are summarized in supplementary table 1 and 2.

Statistical analysis

All results were expressed as mean with standard errors of the mean (SEM). A student's two-tailed *t* test was used when comparing two sets of data. $p < 0.05$ was considered statistically significant. For life span, log-rank tests were performed for survival curves by the Prism 4 software. $p < 0.001$ was considered statistically significant.

Results

PE purification and characterization

Two successive cycles of freezing and thawing at -25 and 4 $^{\circ}\text{C}$, respectively, was found optimum for the extraction of crude intracellular content from the cyanobacterial cells. PE purified from crude extract, was assessed for purity and homogeneity by UV-visible spectroscopy and gel electrophoretic analysis. UV-visible spectrum showing peak at 565 nm dominated over the peak at 280 nm (Fig. 1), indicating the absence of other protein impurities. PE preparation, obtained by described protocol, was found to have purity ratio of about 6.75 (A_{565}/A_{280} , calculated from UV-visible spectrum). Moreover, manifestation of only a single band on the native-PAGE of purified PE stated the purity as well as homogeneity of PE preparation (Fig. 1 inset).

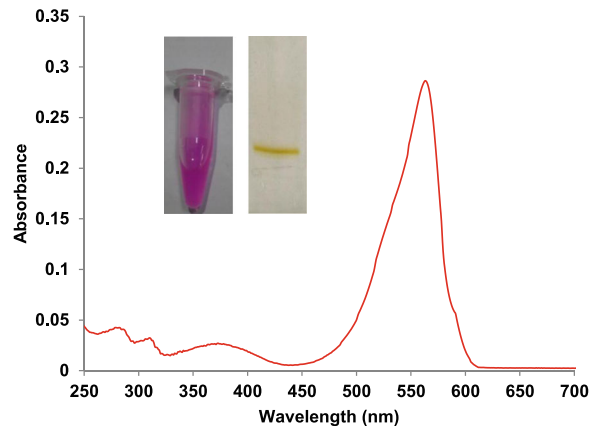


Fig. 1 PE purification and characterization. UV-visible absorbance spectrum of purified PE from *Lyngbya* sp. A09DM. Purified PE and its native-PAGE profile upon silver staining (*Inset*)

PE treatment increases life span and delays signs of aging in *C. elegans*

Effect of PE on life span of *C. elegans* was examined by growing the L4 animals at 20 $^{\circ}\text{C}$ under different PE concentrations (0—control, 10, 50, and 100 $\mu\text{g}/\text{ml}$). The number of dead animals were scored every second day from young adult stage till complete death of all the animals. The mean life span of control animals was found to be 15 ± 0.1 (mean life span \pm standard error of mean) days compared to PE-treated animals showing respective values of 15 ± 0.4 days at 10 $\mu\text{g}/\text{ml}$ PE 17.3 ± 0.1 days for 50 $\mu\text{g}/\text{ml}$ PE, and 19.9 ± 0.3 days for 100 $\mu\text{g}/\text{ml}$ PE concentrations, respectively ($p < 0.001$, log-rank test) (Fig. 2a). PE treatment showed a significant increase in the life span in a dose-dependent manner; however, the animal's brood size and health did not show any difference (data not shown).

To check the efficacy of PE in delaying aging, we measure surrogate physiological markers of aging like pharyngeal pumping rate and rate of worm locomotion in PE-treated (100 $\mu\text{g}/\text{ml}$) and untreated condition in the time course of aging (5th and 10th day post adulthood). The data is represented as mean \pm standard error of mean. The rate of pharyngeal pumping for control animals was found to be 120 ± 15.5 and 37 ± 6.2 min^{-1} for 5th and 10th day, respectively compared to PE-treated animals with respective values of 168 ± 19.5 ($p < 4.5 \times 10^{-11}$, *t* test) and 57.2 ± 11.2 min^{-1} ($p < 3.1 \times 10^{-9}$, *t* test) (Fig. 2b). Effect of PE (100 $\mu\text{g}/\text{ml}$) treatment on worms locomotory behavior with aging was assayed by counting the number of body bends per minute. The

Fig. 2 PE treatment extends life span and improves health span of *C. elegans*. **a** Effect of PE treatment on mean life span. PE pre-exposure improved the N2 adult's survival in dose-dependent manner. Mean survival time—Control, 15 ± 0.1 (mean \pm SEM) days; PE (10 $\mu\text{g/ml}$), 15 ± 0.4 days; PE (50 $\mu\text{g/ml}$), 17.3 ± 0.1 days; PE (100 $\mu\text{g/ml}$), 19.9 ± 0.3 days. $p < 0.001$ (log-rank test). Data is represented as mean \pm SEM. **b** PE treatment delays the progression of decline in pharyngeal pumping rate with aging. Effect of PE on the pharyngeal pumping of *C. elegans* at 5th and 10th day post adulthood in N2 animals. Pharyngeal pumping at 5th day—control, $120 \pm 15.5 \text{ min}^{-1}$, number of animals=165; PE treated (100 $\mu\text{g/ml}$), $168 \pm 19.5 \text{ min}^{-1}$, number of animals=152 ($***p < 4.5 \times 10^{-11}$, *t* test). Pharyngeal pumping at 10th day—control, $37 \pm 6.2 \text{ min}^{-1}$, number of animals=110; PE treated (100 $\mu\text{g/ml}$), $57.2 \pm 11.2 \text{ min}^{-1}$, number of animals=145 ($***p < 3.1 \times 10^{-9}$, *t* test). Data is represented as mean \pm SEM. **c** PE treatment delays progression of locomotion decline with aging. Average body bends per minute in control and PE-treated *C. elegans* scored at 5th and 10th day post adulthood in N2 animals. Body bends per minute—control, 100 ± 5.8 (at 5th day) and 18.00 ± 3.2 (at 10th day); PE treated (100 $\mu\text{g/ml}$), 111 ± 4.6 ($**p < 5.1 \times 10^{-9}$, *t* test) (at 5th day) and 29 ± 4.4 ($**p < 2.3 \times 10^{-10}$, *t* test) (at 10th day). Data represented as mean \pm SEM

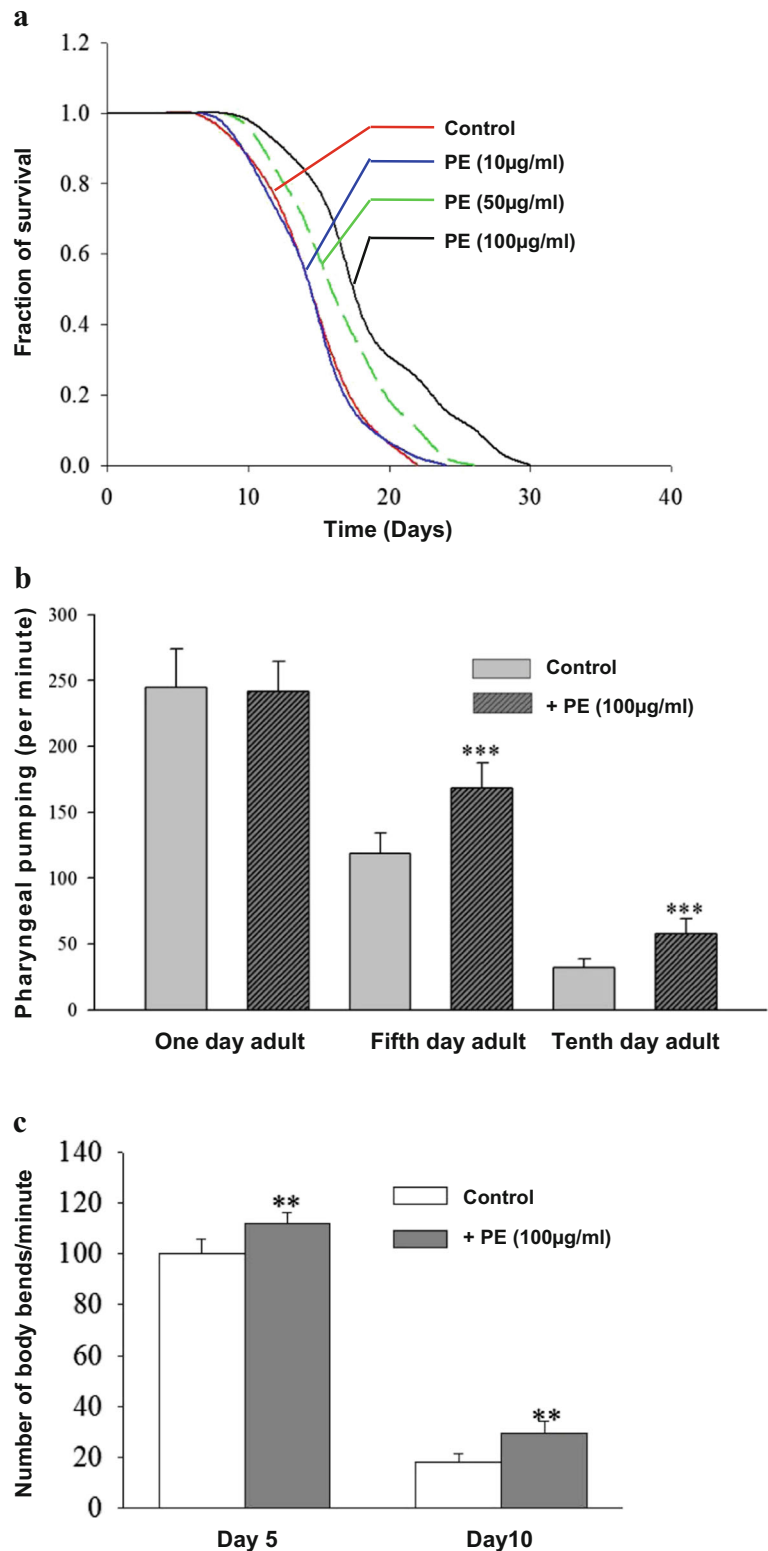


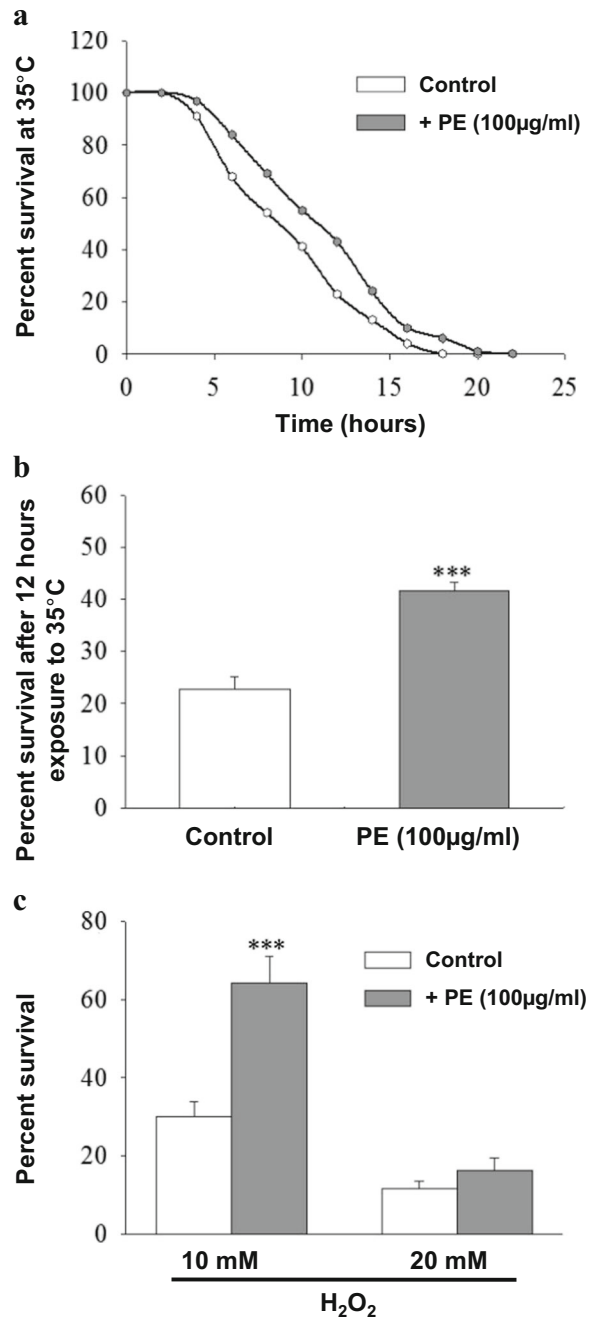
Fig. 3 PE treatment-enhanced stress tolerance in *C. elegans*. **a** PE treatment-enhanced thermo stress tolerance. N2 animals (4th day post adulthood) with and without PE pretreatment of 100 $\mu\text{g/ml}$ were transferred to 35 °C for 12 h. The number of dead animals were scored every 2 h until the 12th hour and depicted as survival curves. **b** PE treatment-enhanced thermo stress tolerance. Mean survival percentages of PE-treated and untreated N2 animals (4th day post adulthood) subjected to 35 °C for 12 h. Mean survival time—untreated control, 22.2 \pm 2.5 %; PE treated (100 $\mu\text{g/ml}$), 41.6 \pm 2.5 % (** p <0.0001, t test). Data is represented as mean \pm SEM. **c** PE treatment-enhanced oxidative stress tolerance. PE-treated and untreated N2 animals (4th day post adulthood) exposed to 10 and 20 mM hydrogen peroxide for 2 h at 20 °C. Survival percentage of animals was scored 16 h post recovery from oxidative stress. Mean survival percentage at 10 mM H_2O_2 exposure is 63.1 \pm 6.4 (control) and 30.1 \pm 3.2 % (PE-treated) (** p >0.0001, t test). Mean survival percentage at 20 mM H_2O_2 exposure is 10.1 \pm 0.9 (control) and 11.5 \pm 1.3 (PE-treated). Data is represented as mean \pm SEM

number of body bends per minute observed for control animals were 100 \pm 5.8 and 18.00 \pm 3.2 for 5th and 10th day, respectively compared to PE (100 $\mu\text{g/ml}$) treated with 111 \pm 4.6 (p <5.1 \times 10⁻⁹, t test) and 29 \pm 4.4 (p <2.3 \times 10⁻¹⁰, t test) values for 5th and 10th day, respectively (Fig. 2c). PE-treated worms showed significantly better pharyngeal pumping rate and locomotory response compared to control worms. Results implied that PE treatment attenuated the aging related depreciation in physiological functions such feeding and locomotion to a certain extent.

PE treatment enhances stress tolerance in *C. elegans*

To explore the protective role of PE against stress, we assayed PE-treated and untreated worms for heat and oxidative stress resistance. Assay plates were prepared by culturing N2 young adult worms in PE-treated (100 $\mu\text{g/ml}$) and untreated plates for 4 days at 20 °C. For thermo tolerance assay, both PE-treated and untreated worms were shifted to 35 °C and numbers of dead worms were scored at every 2 h interval until the 12th hour. Worms pre-exposed with 100 $\mu\text{g/ml}$ PE showed significantly higher survival rate compared to untreated worms (Fig. 3a). The mean survival percentage at 35 °C in time span of 12 h was found to be significantly higher for PE-treated (100 $\mu\text{g/ml}$) worms with mean value of 41.6 \pm 2.5 % compared to control animals 22.2 \pm 2.5 % (p <0.0001, t test) (Fig. 3b).

Animals were subjected to oxidative stress by treating them with H_2O_2 (10 and 20 mM) for 2 h and



were scored 16 h post recovery. PE-treated (100 $\mu\text{g/ml}$) animals subjected to 10 mM H_2O_2 showed significantly higher mean survival percentage compared to control with the respective value of 63.1 \pm 6.4 % (PE-treated, p <0.0001, t test) and 30.1 \pm 3.2 % (untreated control). However, no significant difference was observed between PE-treated

and untreated control animals when exposed to 20 mM H₂O₂ (Fig. 3c). Altogether, these results show that PE treatment confers enhanced stress tolerance in *C. elegans*.

Extension of life span by PE is independent of DAF-2, AGE-1, and DAF-16 signaling

DAF-16 is major effector of IGF-1/insulin signaling pathway, where upstream signaling cascade like DAF-2/AGE-1 result in phosphorylation of DAF-16 resulting in blockage of its nuclear translocation (Ogg et al. 1997; Murphy and Hu 2013). Mutation in *daf-2/age-1* permits nuclear translocation of DAF-16 resulting in activation of genes responsible for life span extension and stress resistance (Lin et al. 1997; Ogg et al. 1997; Murphy and Hu 2013). DAF-16 is expressed in the cytoplasm and move into the nucleus to regulate expression of various stress response gene. To check the effect of PE on IGF-1 signaling pathway, we looked at DAF-16 protein expression in PE-treated and untreated *C. elegans* by probing the nuclear localization of DAF-16 using DAF-16::GFP transgenic animals. Nuclear localization of DAF 16::GFP was induced by subjecting DAF 16::GFP animals to heat shock (35 °C) for 15 min. DAF-16::GFP transgenic animals were found to show more stress tolerance and longevity upon PE treatment irrespective of DAF-16::GFP expression and localization. No significant change in the expression of DAF-16::GFP upon PE treatment was observed, ruling out the role of the DAF-16 pathway in PE-mediated life span extension (Fig. 4a). The same was also examined by observing the effect of PE (100 µg/ml) treatment on the life span of *daf-16* null mutant strains, *mu86*. PE treatment showed increase in the life span of *daf-16(mu86)* animals with the mean value of 16.6±1.2 days (mean life span±standard error of mean), compared to untreated control worms, 12.8±0.8 days ($p<0.001$, log-rank test) (Fig. 4b). Results thus showed that the life span enhancement by PE treatment is independent of DAF-16, a downstream molecule of insulin/ IGF-1 signaling pathway.

We also checked the effect of PE treatment on upstream components like DAF-2/IGF-1 receptor (IGFR) and AGE-1/phosphoinositide 3-kinase (PI3K) of IGF-1 signaling pathways. DAF-2 is *C. elegans* homolog for mammalian IGF-1 receptor and is the only IGF-1/insulin signaling receptor present in *C. elegans* whereas AGE-1 is homolog for mammalian PI3K catalytic

subunit (Finch and Ruvkun 2001). Mutants of both *age-1* and *daf-2* have been reported to result in increased life span (Samuelson et al. 2007; Murphy and Hu 2013). PE treatment enhanced the life span significantly for both *daf-2(e1370)* and *age-1(hx546)* mutant strains with mean survival time±standard error of mean value of 35.2±1.3 days ($p<0.001$, log-rank test) (untreated control—30.1±2.1 days) for *daf-2(e1370)* and 35.4±2.6 days ($p<0.001$, log-rank test) (untreated control—29.5±1.6 days) for *age-1(hx546)*, respectively (Fig. 4c, d).

To examine further, we also checked the expression of SOD-3, one of the direct targets of DAF-16. No considerable change in expression of SOD-3::GFP expression was observed on PE addition (sup figure 1). To examine the role of dietary restriction (DR) on PE-dependent enhancement in life span, we performed the life span assay in *eat-2(ad1116)* mutants. Mutation in *eat-2* gene causes the reduced pharyngeal pumping rate which results in lesser food intake (Crawford et al. 2007). The mean life span of *eat-2(ad1116)* increased from 15.9±0.9 to 18.8±1.1 days ($p<0.001$, log-rank test) upon PE treatment (100 µg/ml) (sup figure 2), suggesting that the PE-mediated life span expansion is independent of DR. Results thus show that PE treatment effects are DAF-2, AGE-1, and DAF-16 independent.

PE ameliorates protein aggregation and prototoxicity-mediated paralysis phenotype

PE is a potent antioxidant (Soni et al. 2009), and numerous lines of evidence have reported involvement of oxidative stress in occurrence and progression of Alzheimer's disease and Aβ toxicity (Jomova et al. 2010). We analyzed the effect of PE on Aβ aggregation using *C. elegans* CL4176, a transgenic model for Alzheimer's disease. The strain CL4176 (*dvIs27[pAF29(myo-3/Ab 1-42/let UTR)+pRF4(rol-6(su1006))]*) is engineered to provide temperature-inducible muscle expression of a human β-amyloid peptide (Aβ) transgene, resulting in paralysis phenotype due to Aβ toxicity upon temperature upshift to 25 °C (Link 2003). Synchronized eggs from transgenic worms were seeded on NGM plates with (100 µg/ml PE) and without PE and incubated for 36 h at 16 °C. This was followed by temperature upshift to 22 and 25 °C, respectively to induce Aβ expression. Worms that did not move or only moved their head when gently touched by a platinum loop were scored as paralyzed. A time course

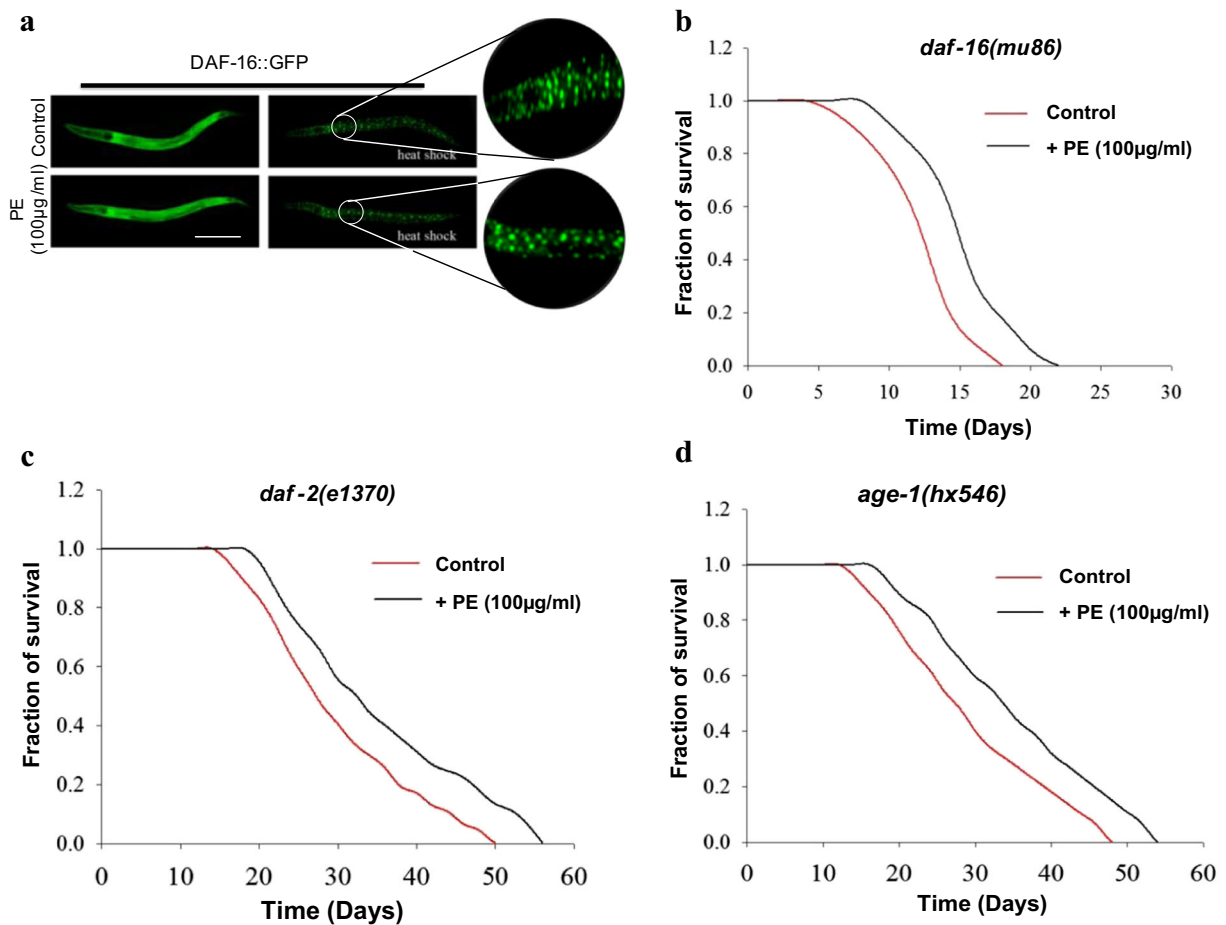


Fig. 4 Effect of PE on life span is independent of DAF-2/AGE-1/DAF-16. **a** Representative image showing nuclear localization of DAF 16::GFP. DAF 16::GFP animals were grown in PE-treated and untreated conditions and subjected to heat shock (35 °C) for 15 min to induce nuclear localization of DAF 16::GFP. Scale bar represents 200 µm. **b** Effect of PE treatment on life span of *daf-16(mu86)* mutant. PE exposure increased the mean life span of *daf-16(mu86)* mutant indicating PE effects are DAF-16 independent. Mean life span—control, 12.8±1.2 days; PE-treated (100 µg/ml), 16.6±0.4 days ($p<0.001$, log-rank test). Data is represented as mean±SEM. **c** Effect of PE treatment on life span

of *daf-2(e1370)* mutant. PE exposure increased the mean life span of *daf-2(e1370)* mutant indicating PE effect is independent of DAF-2/IGF-1 receptor. Mean life span of control animals, 30.1±0.9 days; PE-treated (100 µg/ml), 35.2±1.3 days ($p<0.001$, log-rank test). Data is represented as mean±SEM. **d** Effect of PE treatment on life span of *age-1(hx546)* mutant. PE exposure increased the mean life span of *age-1(hx546)* mutant indicating PE effect is independent of AGE-1—the key kinase of the IGF-1 pathway. Mean life span of control animals, 29.5±1.5 days; PE-treated (100 µg/ml), 35.4±1.5 days ($p<0.001$, log-rank test). Data is represented as mean±SEM

of paralysis for CL4176 worms was recorded post temperature upshift for both 22 and 25 °C for total duration of 36–48 h. A significant delay in paralysis phenotype was observed in the PE-treated worms compared with untreated control (Fig. 5a). The reproducibility of paralysis at a given temperature (22 or 25 °C) in individual trials was consistent. We also quantified paralysis time, defined as time interval from the onset of paralysis to the time at which 50 % of the worms were paralyzed. A statistically significant decrease in A β -induced

paralysis and delay in paralysis time was observed in the worm fed with PE (Fig. 5b, c).

PE-mediated effects are dependent on HSF-1

HSF-1 and SKN-1 transcriptional factors have been also reported to be involved in insulin/IGF-1 signaling in response to cellular stress (Chiang et al 2012; Morton and Lamitina 2013; Tullet et al 2008). To investigate whether the protective effect of PE is mediated through

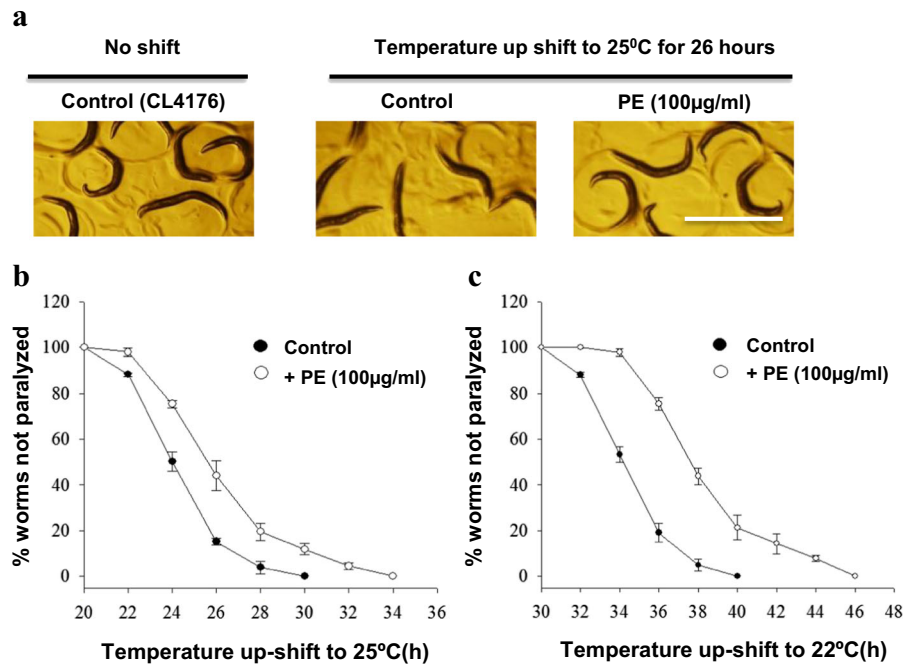


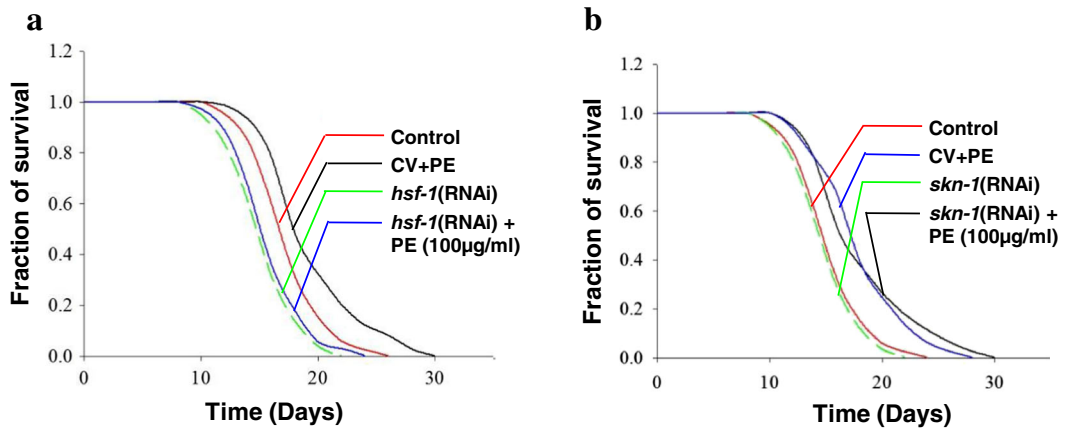
Fig. 5 PE is effective in preventing protein aggregation and delaying prototoxicity-mediated paralysis phenotype in CL4176 animals. **a** Representative image of CL4176 transgenic animals showing paralysis due to A β expression. Representative images of control (no temperature shift) at 16 °C and effect of temperature upshift to 25 °C for induction of A β expression in control and PE-

treated (100 μ g/ml) CL4176 transgenic animals. *Scale bar* represents 1 mm. **b–c** Effect of PE in suppressing A β _{1–42}-associated paralysis phenotype in CL4176 animals. Survival curves (in term of paralysis) after temperature upshift to 25 °C (**b**) and 22 °C (**c**) are represented which show significant increase in survival percentage. Data points are represented as mean \pm SEM

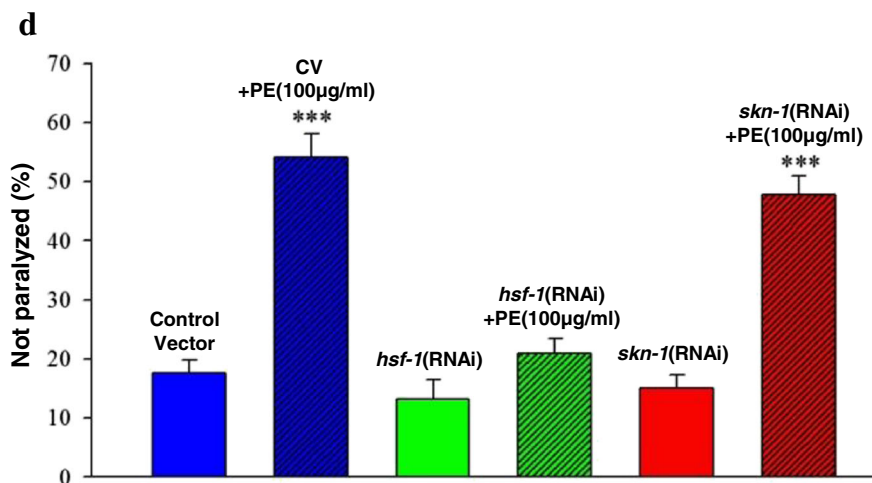
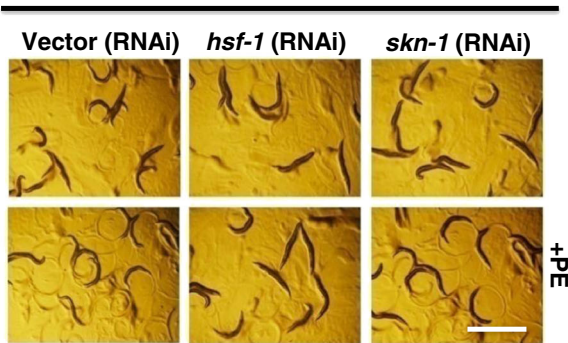
transcriptional factor for heat shock (HSF-1) and oxidative stress (SKN-1) responses, we performed the life span and paralysis assay in wild type and CL4176 animals, respectively in PE-treated (100 μ g/ml) and untreated conditions when HSF-1 and SKN-1 genes were knocked out using RNAi. The mean life span \pm SEM for control animals was 15.9 \pm 1.2 days; *hsf-1* (RNAi) animals, 13.7 \pm 1.4 days; and *skn-1* (RNAi) animals, 15.5 \pm 1.6 days compared with PE-treated control animals, 18.6 \pm 1.3 days (p <0.001, log-rank test); *hsf-1* (RNAi) animals, 14.3 \pm 1.5 days (p =0.33, log-rank test); and *skn-1* (RNAi) animals, 18.5 \pm 1.9 days, (p <0.001, log-rank test), respectively (Fig. 6a, b). There was no increment in life span on PE treatment when HSF-1 was knockdown; in contrast, there was significant increase in life span when SKN-1 was knockdown. To confirm the dependency of the life span expansion effect of PE on HSF-1, we examined *hsf-1* mRNA levels in N2 Bristol upon PE treatment (100 μ g/ml). We found around 1.7-fold increase in the mRNA level in PE-treated worms (sup figure 3). Altogether, it showed that

Fig. 6 PE-mediated increase in life span show HSF-1 dependence. ►

a Effect of PE treatment on life span of *hsf-1* (RNAi). PE exposure does not increase the mean life span of *hsf-1* (RNAi) mutant, indicating dependency of PE effect on HSF-1. Mean life span—control vector, 15.9 \pm 1.2 days; *hsf-1* (RNAi), 13.7 \pm 1.4 days; control vector+PE, 18.6 \pm 1.3 days (p <0.001, log-rank test); *hsf-1* (RNAi)+PE, 14.3 \pm 1.5 days (p =0.33, log-rank test). Data is represented as mean \pm SEM. **b** Effect of PE treatment on life span of *skn-1* (RNAi). PE exposure increased the mean life span of *skn-1* (RNAi) mutant, indicating PE effects are independent of SKN-1. Mean life span—control vector, 15.9 \pm 1.2 days; *skn-1* (RNAi), 15.5 \pm 1.6 days; control vector+PE, 18.6 \pm 1.3 days (p <0.001, log-rank test); *skn-1* (RNAi)+PE, 18.5 \pm 1.9 days, (p <0.001, log-rank test). Data is represented as mean \pm SEM. **c** Representative images of CL4176 animals showing paralysis at 25 °C when subjected to *hsf-1* and *skn-1* RNAi. PE-treated (100 μ g/ml) and untreated control vector (CV), *hsf-1* (RNAi), and *skn-1* (RNAi) CL4176 *C. elegans* animals subjected to 25 °C. *Scale bar* represents 1 mm. **d** Fraction of non-paralyzed worms PE-treated (100 μ g/ml) and untreated subjected to *hsf-1* and *skn-1* RNAi. Fraction of non-paralyzed CL4176 worms—control vector, 18.1 \pm 2.7; *hsf-1* (RNAi), 15.8 \pm 4.8; *skn-1* (RNAi), 17.7 \pm 2.8; control vector+PE, 55.3 \pm 3.9 (***) p <6.5 \times 10⁻¹¹, *t* test); *hsf-1* (RNAi)+PE, 22.4 \pm 3.5 (p =0.02, *t* test); *skn-1* (RNAi)+PE, 49.2 \pm 4.6 (***) p <2.3 \times 10⁻¹², *t* test). Data represented as mean \pm SEM



c Temperature up shift to 25°C for 26 hours



HSF-1 is required for enhancement of life span mediated by PE treatment.

As HSF-1 and SKN-1 are involved in stress response; we also assayed the effect of HSF-1 and SKN-

1 on A β aggregation. PE-treated CL4176 animals were grown in control and HSF-1 and SKN-1 knockout (RNAi) conditions. Paralysis time was quantified for all the experimental conditions after 36 h of incubation at 25 °C. The mean value of percentage of non-paralyzed worm \pm standard error of mean for control animals was 18.1 ± 2.7 ; *hsf-1* (RNAi) animals, 15.8 ± 4.8 ; and *skn-1* (RNAi) animals, 17.7 ± 2.8 as compared to PE-treated control, 55.3 ± 3.9 ($p < 6.5 \times 10^{-11}$, *t* test); *hsf-1* (RNAi), 22.4 ± 3.5 ($p = 0.02$, *t* test); and *skn-1* (RNAi) 49.2 ± 4.6 ($p < 2.3 \times 10^{-12}$, *t* test) (Fig. 6c, d). Comparative accounts of mean and maximum life span of control and PE-treated *C. elegans* strain in various assays are depicted in supplementary table 2. Altogether, these results depicts that PE-dependent increase in life span requires HSF-1.

Discussion

Aging pattern between *C. elegans* and mammals has considerable degree of similarity. *C. elegans* has thus turned out to be a potential model organism for aging-related studies to explore potential anti-aging drugs. ROS has been reported as a major cause of aging, and antioxidants have been reported to curtail the aging effects of ROS. Antioxidants reduce the extent of free radicals-mediated oxidative damage and hence results in increased life span and healthy aging. Several studies are being undertaken to explore possible anti-aging wonder drugs. We checked the potency of PE as possible candidate for curtailing aging and age-related disorders. Our results show that exposure to PE which is a chromophore-containing pigment protein, isolated from cyanobacterium *Lyngbya* sp. A09DM, increases life span and health span of *C. elegans*. Various physiological functions including pharyngeal pumping, locomotory health span, feeding rate, and sarcopenia in *C. elegans* are considered as the hallmarks of aging (Huang et al. 2004). The beneficial effect of PE feeding on these hallmarks of aging in *C. elegans* also revealed the role of PE in improving health span with increasing age. Efficiency of PE in averting the A β -peptide mediated paralysis was probed by using CL4176 (*C. elegans* model for Alzheimer's disease). CL4176 was constructed by mutating the *smg-1* gene (involved in nonsense-mediated mRNA decay) for heat-induced overexpression of A β -peptide in their muscle cells, which in turn

initiated the oxidative stress-mediated neurotoxicity manifested in paralysis phenotype upon temperature upshift (Dostal and Link 2010). PE treatment was found to delay A β -peptide-mediated paralysis, indicating the importance and function of PE's antioxidant virtue in its anti-paralytic activity. This result suggested that PE can efficiently obviate the oxidative stress by its antioxidant virtue. PE treatment also provided tolerance to oxidative as well as thermo stress. These results confirmed that PE treatment enhances life span and promotes healthy aging in *C. elegans*. We also tried exploring the possible pathways through which PE might be showing its effect. One possible mechanism behind the PE effect may be the hormetic effect, which is low dose exposure of toxicant enhances the damage repair mechanism and increases life span consequently (Rattan 2008). PE does not show hormetic effect as it does not show lethality at higher doses. Moreover, toxicity analysis exhibited by Parmar et al., (unpublished), also supports PE as non-toxic and non-lethal and thus rejecting the hormetic effect. The beneficial effect of PE might be due to the inhibition of some of the well conserved mechanisms of aging. IIS signaling components and pathway are highly conserved among *C. elegans* and other mammals. FOXO family transcription factor DAF-16 and their upstream components like DAF-2/IGF-1 receptor (IGFR) and AGE-1/ phosphoinositide 3-kinase (PI3K) are well established as central regulatory elements for longevity and stress-associated responses. Null mutants for *daf-16(mu86)*, *daf-2(e1370)*, and *age-1(hx546)* cultured under PE treatment showed significant expansion in life span, indicating that PE works independently of these IIS signaling elements. Moreover the expression of DAF-16 in DAF-16::GFP transgenic animal upon PE treatment showed no significant difference in nuclear localization of the DAF-16::GFP protein. All results collectively proved that PE effects are independent of the DAF-2–AGE-1–DAF-16 pathway. SKN-1 is an important component for oxidative stress responses and is required for life span extension by dietary restriction or inhibition of the TORC1 or TORC2 pathways (Robida-Stubbs et al. 2012). Results of PE effect on null mutants for SKN-1 ruled out the involvement of TOR pathways inhibition in extending life span on PE treatment.

On the contrary, HSF-1 controls stress-induced gene expression is found to be essential for PE-mediated life expansion. The recent work of Chiang et al. (2012) showed that HSF-1 activity is being regulated by IIS via formation of a multi-molecular complex containing

the conserved proteins DDL-1 and DDL-2 that sequesters HSF-1 in the cytoplasm. Our results show that the PE-mediated effect is HSF-1 dependent but IIS independent. This may be due to some parallel alternative process may be in hold for HSF-1 activity along with the IIS pathway. The exact signaling pathway and mechanism by which PE mediates its effect is still unclear. Though, some possible routes, by which PE may work, are ruled out in the present experimental work. The effects demonstrated by PE on longevity and aging in *C. elegans* led us to hypothesize that PE is a potent antioxidant and shows beneficial effect on aging and age-related disorders.

Conclusion

The findings of this report reveal a novel role of PE in extending life span and health span in *C. elegans*. Our results show that PE supplement not only increases life span but also improves quality of life by delaying aging effects like declining locomotion and feeding rate. It also enhances the stress tolerance capacity and decreases A β -induced paralysis in *C. elegans* Alzheimer's model. We also checked the signaling pathway through which PE treatment would be affecting. As per our results, life span-prolonging activity of PE does not seem to be associated with well conserved IIS pathway, as PE functions even in the absence of some of the IIS pathway components. However, presence of HSF-1 seems to be important in mediating anti-aging effects of PE. Our results using the *C. elegans* model gives quite strong evidence for PE as a promising anti-aging candidate. Furthermore, research using other model systems needs to be carried out in the future to establish the potential of PE as an anti-aging wonder drug.

Acknowledgments This research was supported by the following funding agencies: (1) the Department of Science and Technology DST and (2) DBT-Patna University-IPLS Programme funded by the Department of Biotechnology (DBT), Govt. of India. RRS gratefully acknowledges the Department of Science and Technology (DST), New Delhi for financial support in the form of IN-SPIRE fellowship.

References

- Benedetti MG, Foster AL, Vantipalli MC, White MP, Sampayo JN, Gill MS, Olsen A, Lithgow GJ (2008) Compounds that confer thermal stress resistance and extended lifespan. *Exp Gerontol* 43:882–891
- Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *J Am Med Assoc* 297:842–857
- Blagosklonny MV (2008) Aging: ROS or TOR. *Cell Cycle* 7: 3344–3354
- Brenner S (1974) The genetics of *Caenorhabditis elegans*. *Genetics* 77:71–94
- Brown MK, Evans JL, Luo Y (2006) Beneficial effects of natural antioxidants EGCG and alpha-lipoic acid on life span and age-dependent behavioral declines in *Caenorhabditis elegans*. *Pharmacol Biochem Behav* 85:620–628
- Cai WJ, Huang JH, Zhang SQ, Wu B, Kapahi P, Zhang XM, Shen ZY (2011) Icaritin and its derivative icariside II extend healthspan via insulin/IGF-1 pathway in *C. elegans*. *PLoS ONE* 6:e28835
- Chiang WC, Ching TT, Lee HC, Mousigian C, Hsu AL (2012) HSF-1 regulators DDL-1/2 link insulin-like signaling to heat-shock responses and modulation of longevity. *Cell* 148:322–334
- Cian RE, López-Posadas R, Drago SR, de Medina FS, Martínez-Augustín O (2012) Immunomodulatory properties of the protein fraction from *Phorphyra columbina*. *J Agric Food Chem* 60:8146–8154
- Crawford D, Libina N, Kenyon C (2007) *Caenorhabditis elegans* integrates food and reproductive signals in lifespan determination. *Aging Cell* 6:715–721
- Dostal V, Link CD (2010) Assaying β -amyloid Toxicity using a Transgenic *C. elegans* Model. *JoVE*. 44
- Finch CE, Ruvkun G (2001) The genetics of aging. *Annu Rev Genomics Hum Genet* 2:435–462
- Gems D, Doonan R (2009) Antioxidant defense and aging in *C. elegans*: is the oxidative damage theory of aging wrong? *Cell Cycle* 8:1681–1687
- Harman D (1998) Aging and oxidative stress. *J Int Fed Clin Chem* 10:24–27
- Harrington LA, Harley CB (1988) Effect of vitamin E on lifespan and reproduction in *Caenorhabditis elegans*. *Mech Aging Dev* 43:71–78
- Huang C, Xiong C, Kornfeld K (2004) Measurements of age-related changes of physiological processes that predict lifespan of *Caenorhabditis elegans*. *Proc Natl Acad Sci* 101:8084–8089
- Ishii N, Senoo-Matsuda N, Miyake K, Yasuda K, Ishii T, Hartman PS, Furukawa S (2004) Coenzyme Q10 can prolong *C. elegans* lifespan by lowering oxidative stress. *Mech Aging Dev* 125:41–46
- Iwasa H, Yu S, Xue J, Driscoll M (2010) Novel EGF pathway regulators modulate *C. elegans* healthspan and lifespan via EGF receptor, PLC- γ , and IP3R activation. *Aging Cell* 9: 490–505
- Jomova K, Vondrakova D, Lawson M, Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* 345:91–104
- Kamath RS, Ahringer J (2003) Genome-wide RNAi screening in *Caenorhabditis elegans*. *Methods* 30: 313–321
- Kenyon CJ (2010) The genetics of aging. *Nature* 464:504–512

- Kenyon C, Chang J, Gensch E, Rudener A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366:461–464
- Kumar J, Choudhary BC, Metpally R, Zheng Q, Nonet ML, Ramanathan S, Klopfenstein DR, Koushika SP (2010) The *Caenorhabditis elegans* Kinesin-3 motor UNC-104/KIF1A is degraded upon loss of specific binding to cargo. *PLoS Genet* 6:e1001200
- Lin K, Dorman JB, Rodan A, Kenyon C (1997) daf-16: An HNF-3/fork head family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* 278:1319–1322
- Link C (2003) Gene expression analysis in a transgenic *Caenorhabditis elegans* Alzheimer's disease model. *Neurobiol Aging* 24:397–413
- Lordan S, Ross RP, Stanton C (2011) Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Mar Drugs* 9:1056–1100
- Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ (2000) Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 289:1567–1569
- Morton EA, Lamitina T (2013) *Caenorhabditis elegans* HSF-1 is an essential nuclear protein that forms stress granule-like structures following heat shock. *Aging Cell* 12:112–120
- Murphy CT, Hu PJ (2013) Insulin/insulin-like growth factor signaling in *C. elegans*. *WormBook: the online review of C. elegans biology*, 1
- Narasimhan SD, Yen K, Tissenbaum HA (2009) Converging pathways in lifespan regulation. *Curr Biol* 19:R657–R666
- Ogg S, Paradis S, Gottlieb S, Patterson GI, Lee L, Tissenbaum HA, Ruvkun G (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* 389:994–999
- Parmar A, Singh NK, Kaushal A, Sonawala S, Madamwar D (2011) Purification, characterization and comparison of phycoerythrins from three different marine cyanobacterial cultures. *Bioresour Technol* 102:1795–1802
- Rasmussen RS, Morrissey MT (2007) Marine biotechnology for production of food ingredients. *Adv Food Nutr Res* 52:237–292
- Rattan SI (2008) Hormesis in aging. *Aging Res Rev* 7:63–78
- Robida-Stubbs S, Glover-Cutter K, Lamming DW, Mizunuma M, Narasimhan SD, Neumann-Haefelin E, Sabatini DM, Blackwell TK (2012) TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metab* 15:713–724
- Romay CH, Gonzalez R, Ledon N, Ramirez D, Rimbau V (2003) C-phycoerythrin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 4:207–216
- Samuelson AV, Carr CE, Ruvkun G (2007) Gene activities that mediate increased life span of *C. elegans* insulin-like signaling mutants. *Genes Dev* 21:2976–2994
- Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 6:280–293
- Shah V, Garg N, Madamwar D (2001) Record of the marine cyanobacterium from the rocky shores of Bet-Dwarka and Okha, India. *Acta Bot Mal* 26:188–193
- Singh NK, Hasan SS, Kumar J, Raj I, Pathan AA, Parmar A, Shakil S, Gourinath S, Madamwar D (2014) Crystal structure and interaction of phycoerythrin with β -Secretase: a putative therapy for Alzheimer's disease. *CNS Neurol Disord Drug Targets* 13:691–698
- Sonani RR, Singh NK, Kumar J, Thakar D, Madamwar D (2014) Concurrent purification and antioxidant activity of phycobiliproteins from *Lyngbya* sp. A09DM: an antioxidant and anti-aging potential of phycoerythrin in *Caenorhabditis elegans*. *Process Biochem*. doi:10.1016/j.procbio.2014.06.022
- Soni B, Trivedi U, Madamwar D (2008) A novel method for single step hydrophobic interaction chromatography for the purification of phycoerythrin from *Phormidium fragile* and its characterization for antioxidant property. *Bioresour Technol* 99:188–194
- Soni B, Visavadiya NP, Madamwar D (2009) Attenuation of diabetic complications by C-phycoerythrin in rats: antioxidant activity of C-phycoerythrin including copper-induced lipoprotein and serum oxidation. *Br J Nutr* 102:102–109
- Taboada C, Millán R, Míguez I (2010) Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *J Sci Food Agric* 90:445–449
- Timmons L, Fire A (1998) Specific interference by ingested dsRNA. *Nature* 395:854–854
- Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, Liu S, Oliveira RP, Baumeister R, Blackwell TK (2008) Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signalling in *C. elegans*. *Cell* 132:1025–1038
- Vadiraja BB, Giakwad NW, Madyastha KM (1998) Hepatoprotective effect of C phycoerythrin: protection for carbon tetrachloride and R-(+)-pulegone-mediated hepatotoxicity in rats. *Biochem Biophys Res Commun* 249:428–431
- Waterbury JB, Stanier RY (1981) Isolation and growth of cyanobacteria from marine and hypersaline environments—the prokaryotes. Springer, Berlin Heidelberg, pp 221–223
- Zhang LX, Cai CE, Guo TT, Gu JW, Xu HL, Zhou Y, Wang Y, Liu CC, He PM (2011) Anti-cancer effects of polysaccharide and phycoerythrin from *Porphyrta Yezoensis*. *J Mar Sci Tech* 19:377–382
- Zhou KI, Pincus Z, Slack FJ (2011) Longevity and stress in *Caenorhabditis elegans*. *Aging (Albany NY)* 3:733