Chapter 4

Evaluating the long term protective efficacy of PQQ producing probiotic *Escherichia coli* Nissle 1917 strain in aging rats

4.1 Introduction

Life on earth started in anaerobic environment and progressed towards aerobic. The complexities of organisms increased and they started utilizing oxygen to fulfil their energy requirements. The emergence of oxidative phosphorylation brought about the bitter consequence of ROS and oxidative damages where persistent and prolonged presence of free radicals overcomes the cellular defence and leads to senescence. As described by Masoro, (1995) aging is defined as those "***deteriorative changes with time during post-maturational life that underlie an increasing vulnerability to challenges, thereby decreasing the ability of the organism to survive".*** Harmann, another pioneer in the field of gerontology, had described ageing as the progressive "accumulation of deleterious changes in the cells and tissues with the advancement of age, which increase the risk of disease and death" (Harman, 1992). Several theories have come up over the years and have tried to define the basis of ageing. However, all of them directly or in directy fall back upon the same concept of ROS induced oxidative stress, mitochondrial dysfunction and the increment in the oxidative stress with progress in ageing. Mitochondria is involved in the generation of ROS during normal metabolism and this function is corelated with various disorders including aging (Lenaz et al, 2000). Mitochondrial theory of aging postulates that mitochondria, being both, producers and target of ROS, accumulate random mutation in mtDNA which is responsible for energetic decline followed by senescence. Damaged mitochondria initiate vicious cycle leading to the cumulative deleterious effects on cellular processes (Wallace, 2005).

Enormous amount of work on remedial effects of antioxidant therapies have been carried out, however, they had moderate effectiveness or inconclusive results (Kamel et al, 2006). Pyrroloquinoline Quinone (PQQ) is a bacterial redox cofactor secreted by few gram negative bacteria (Rucker et al., 2009). It is one of the most potent antioxidant known till date. This water soluble and heat resistant molecule have been found in almost all plant products, mammalian organs and human milk (Kumazawa et al, 1992; Mitchel et al, 1999). Interestingly, it has been shown to possess more important and

diverse functions in mammals (Killgore et al., 1989). It is believed to be nutritionally essential, and its defeciency hampers normal growth and development. Since it is not synthesized by humans or human gut microbiome, diet is the only source of this molecule. In addition to the antioxidant property, PQQ is known to interact with cell signaling molecules in mammalian cells leading to upregulation of genes involved in mitochondrial biogenesis and metabolism (Kumazawa et al, 2007; Tchaparian et al, 2010). PQQ induces mitochondriogenesis and upregulates cellular metabolism in aging cells as well as in rodent models (Chowanadisai et al, 2010). It also protects neurons and associated pathophysiology (Sanchez et al., 2000; Hara et al, 2007; Zhang et al, 2002; 2009; 2011), prevents DNA damage (Rucker et al., 2009) and regulates lipid metabolism (Baurley et al., 2011). PQQ causes activation of cAMP response element binding protein (CREB) by phosphorylation leading to upregulation of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α), Nuclear Respiratory Factors (NRF-1,2), Tfam, peroxisome peroxisome proliferator-activated receptor-α, carnitine palmitoyl transferase 1 and mitochondrial complex I and II related genes (Chowanadisai et al, 2010). The transcriptional coactivator PGC-1α is key regulator of mitochondrial biogenesis and energy metabolism (Rasbach et al., 2007). Overexpression of PGC-1α prevents cellular injury and promotes mitochondrial recovery in oxidant teated cells. Recent study on human subjects has revealed that PQQ reduces inflammation and improves mitochondrial metabolism (Harris et al., 2013). PQQ also influences the activity of DJ-1, which is invovled in cellular stress response and regulation of apoptosis by inactivating Janus Kinase (JNK) Pathway (Nunome et al., 2008).

Gut mcrobial diversity alters with advancing age and is characterized by decreased bifidobacteria and increased bacteroids species (Hopkins et al., 2002). Alteration in microbial composition with age and disease, alters the metabolic capicity of gut microbiota. Colonic microbial community plays very important role in maintaining normal bowel function and host health through colonization resistance (Round & Mazmanian, 2009), modulation of immune system (Cerf-Bensussan & Gaboriau-Routhiau, 2010) and production of SCFAs (Campbell et al., 1997). Probiotic interventions for restoring normal

microbial composition and function could be an important and effective strategy.

EcN was genetically modified by incorporating PQQ producing operon from and was used as a novel endogenous delivery system by exploiting the potential of probiotic EcN and PQQ (Pandey et al., 2014). Consistant endogenous production and secretion of PQQ in the gut by EcN has been shown to be effective in reducing oxidative stress, mitochondrial dysfunction and altered lipid profile in rats. Present study reports the effect of PQQ secreting EcN on natural ageing rats.

4.2 Methods and materials

4.2.1 Bacterial strains, culture conditions and plasmids.

E. coli Nissle 1917 were maintained at 37 ^{0}C on Luria agar and Luria broth (described in **section2.2**). *E. asburiae* PSI3 was used for extraction of PQQ which was used for feeding to rats. Different bacterial strains and plasmids have been summarized in **Table 4.1**.

Table 4.1 Plasmids and bacterial strains

Plasmids/ Strains	Characteristics	Reference
Plasmids		
pTZ57R/T	Cloning vector Thermo fisher Scientific ®	Thermo fisher Scientific-India ltd. Mumbai.
pTPQQ-1	pTZ57R/T vector harbouring 3.7 Kb *pqq* gene cluster from *G. oxydans*.	This Study (Chapter 3)
Bacterial Strains		
E. coli DH5α	Laboratory strain	Sambrook *et al*., 2002
E. coli Nissle 1917 (EcN)	Probiotic strain	Sonnenborn and Schulze, 2009
EcN-2	EcN strain with genomic integration of *vgb* and *gfp* genes	This study (Chapter 2)
EcN-5	EcN-2 strain harbouring pTPQQ-1 plasmid	This study (Chapter 3)
E. asburiae PSI3	PQQ producing, phosphate solublizing bacteria isolated from pigeon pea rhizosphere	Gyaneshwar *et al*., 1999

4.2.2 Animals

Charles Foster male albino rats 12 months of age were selected for the present study. They were maintained in controlled conditions (temperature: 25 ± 1°C; relative humidity: 45.5 %; photoperiod cycle: 12 h light and 12 h dark) with free access to food and water as per recommendations from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), an animal ethical committee of the institute (The Maharaja Sayajirao University of Baroda, India, Reg. No. 938/A/06/CPCSEA). All rats received regular pellet diet and were granted free access to drinking water.

4.2.3 Designing of the experiment

Aging rats, 12 months of age, were divided in different groups as described in **Figure 4.1**. Streptomycin treatment (5 g/l) was given for 48 h followed by probiotic treatment (10^8 CFU/day) once a week. All groups were treated for 8 months, after which rats were sacrificed and samples were collected for analysis. Regular fecal samples were collected from rats for monitoring presence of respective probiotic EcN strain.

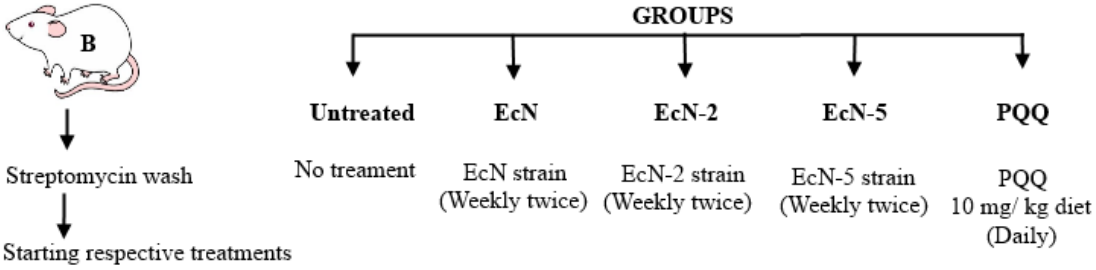

Figure 4.1 Schematic representation of strategy for different animal groups receiving respective treatments in naturally aging rat model.

4.2.4 Enzyme assays and biochemical estimations

Enzyme assays, PQQ extraction and quantification, and other biochemical estimations were performed as described in **section 2.2.** Briefly, Tissue homogenate preparation, enzyme activities (SOD, CAT, Mit-SOD), GSH and lipid peroxidation levels were performed as described elsewhere (Pandey et al., 2014). Lipid content was estimated using kits and following standard protocols (Beacon Diagnostics Pvt. Ltd. Navsari, India). Succinate dehydrogenase (SDH) assay was performed as described earlier (Hopsu and Harkonen, 1959).

4.2.5 mRNA expression and qRT-PCR

Total RNA was extracted from hepatic tissue using TRIzol® (Invitrogen BioServices IndiaPvt. Ltd., Bangalore, India) following manufacturers protocol.

cDNA was generated using Reverse transcriptase kit (Applied Biosystems, Foster City, CA) following standard protocol supplied with the kit. PCR was performed using ABI QuantStudio™ 12L flex real time PCR system coupled with SYBR green technology (Applied Biosystems) following standard cycling parameters. Relative amount of liver mitochondria was determined using real time PCR as described Sites et al. (2006). Nuclear cystic fibrosis (CF) and mitochondrial nicotinamide adenine dinucleotide dehydrogenase (ND-5) were the target genes. Primer sequences are shown in **Table 4.2**.

Table 4.2. Primer sequences used for qRT-PCR

Genes	Sequence
Fatty acid synthase (FAS)	5' ACCTCATCACTAGAAGCCACCAG 3' (Forward) 5' GTGGTACTTGGCCTTGGGTTTA 3' (Reverse)
Acyl coenzyme A oxidase (Acox)	5' ACAAGCTGACGTATGGGACC 3' (Forward) 5' GTGGTTCTGGTTCGCTTTGC 3' (Reverse)
Proliferator-activated receptor-γ coactivator-1α (PGC-1α)	5' AATGAGCCCGCGAACATATT 3' (Forward) 5' TGAGGACCGCTAGCAAGTTTG 3' (Reverse)
Mitochondrial transcription factor A (Tfam)	5' AACGCCTAAAGAAGAAAGCACAA 3' (Forward) 5' CCGAGGTCTTTTTGGTTTTCC 3' (Reverse)
B-actin	5' ACGGTCAGGTCATCACTATCG 3' (Forward) 5' GGCATAGAGGTCTTTACGGATG 3' (Reverse)
Cystic fibrosis (CF)	5' AAACTCAGGATAGCTGTCCGTTTAG 3' (Forward) 5' GCCAAATGATAGCATGGAACTCT 3' (Reverse)
Nicotinamide adenine dinucleotide dehydrogenase (ND-5)	5' GGATGATGATATGGCCTTGCA 3' (Forward) 5' CGACTCGGTTGTAGAGGATTGC 3' (Reverse)

4.2.6 *C. elegans* growth conditions and survival assays

C. elegans experiments and maintenance was generously done by Dr. Neeraj Pandey (S.P. University, Gujarat, India). Strains were grown and maintained as described by Stiernagle et al. (2006).

4.2.7 Statistical analysis

Data analysis was conducted using GraphPad Prism version 5.0 (GraphPad Softwares Inc., San Diego, CA). Results were considered significant at $p \leq 0.05$.

4.3 Results

4.3.1 Antioxidant status and lipid profile of young and aged Charles Foster rats

It is already established that with progressive age, antioxidant capacity of tissues decreases as compared to young tissues. Antioxidant status in blood, liver and colon of 20 months old rats were compared with 4 month young Charles Foster rats. It was found that old rats exhibit significant decrease in antioxidant capacity and increased lipid peroxidation, in tissues analysed, as compared to younger rats (**Figure 4.2**).

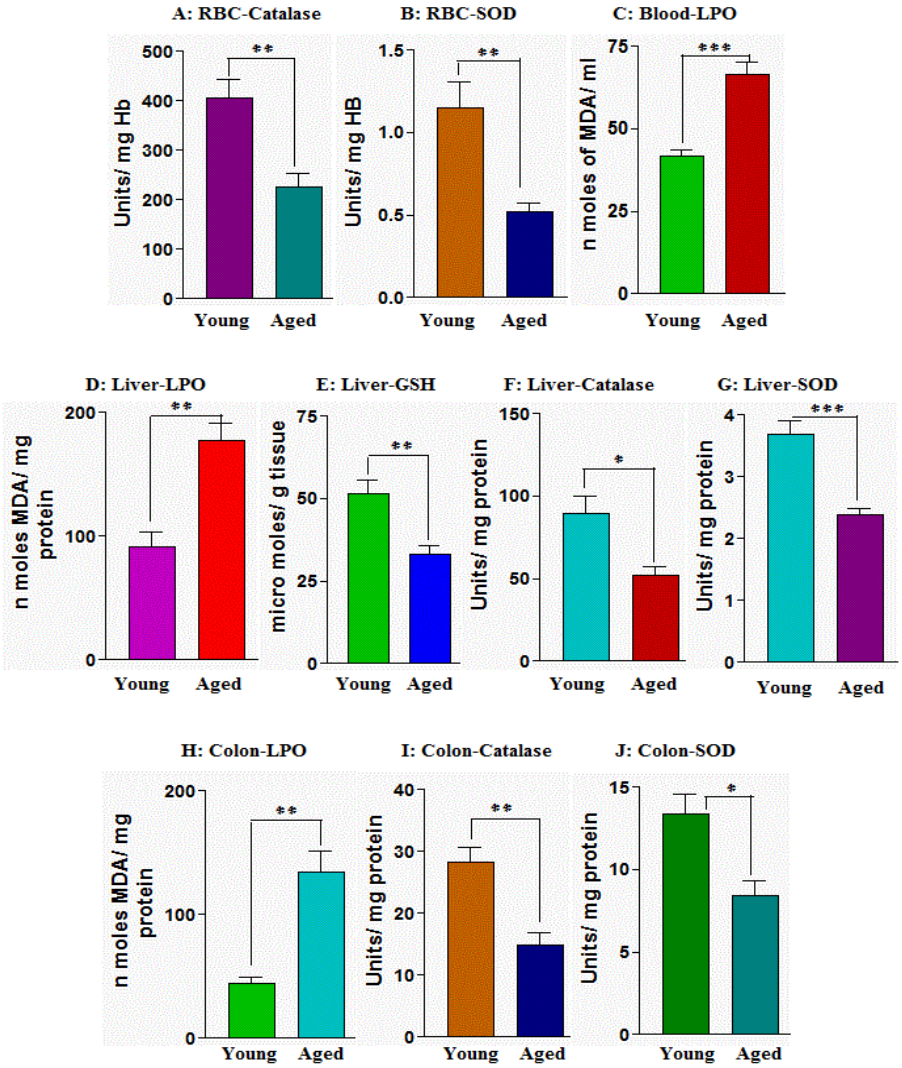

Figure 4.2 Antioxidant status in blood, liver and colon of young and aged Charles Foster rats. All values are represented as mean ± SEM (6 animals per group). *p<0.05, and **p<0.01 represent difference between different groups.

Plasma lipid levels were also found to be altered in old rats as compared to young ones (**Figure 4.3**). This is in consistence with several studies and establishes the same phenomenon in our experimental conditions.

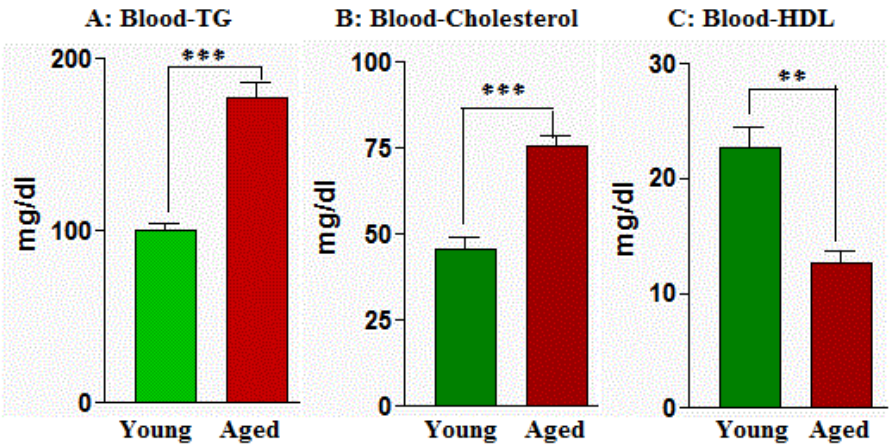

Figure 4.3 Blood lipid profile of young and aged Charles Foster rats. All values are represented as mean ± SEM (6 animals per group). *p<0.05, **p<0.01 and p***<0.001 represent difference between different groups.

4.3.2 Evaluating long term effect of PQQ producing EcN-5 treatment in aging rats

Antioxidant status

Rats fed with EcN-5 and PQQ showed significant reduction in hepatic lipid peroxidation level compared to untreated group (**Figure 4.4 A**). EcN-5 treatment appeared to be more efficient in reducing lipid peroxidation compared to PQQ alone. Moreover, there was significant increase in hepatic GSH level along with CAT and SOD enzyme activities in these rats compared to untreated ones (**Figure 4.4 B-D**). However, no significant difference was found in these parameters among EcN-5 and PQQ treated groups. Importantly, treatment with native EcN and EcN-2 (expressing *vgb* gene) strains had no effect on antioxidant parameters in aging rats.

Interestingly, rats treated with native EcN and EcN-2 strains exhibited significant reduction in colonic oxidative stress in aging rats as indicated by reduction in lipid peroxidation along with elevation of CAT and SOD enzyme activities (**Figure 4.4 D-F**). PQQ alone treated groups also showed similar results. However, incorporation of *pqqABCDE* gene cluster significantly enhanced the efficacy of EcN strains, particularly in colonic tissues, as depicted by more pronounced reduction in oxidative stress parameters in rats treated with EcN-5 strain (**Figure 4.4 D-F**).

Hepatic mitochondrial content and biogenesis

Mt-SOD and SDH activities assessed from mitochondrial fraction of hepatic tissues reveals, EcN-5 treatment positively influences the mitochondrial antioxidant defence and metabolism as depicted by 1.9 fold and 1.5 fold increase in Mt-SOD and SDH activities, respectively compared to untreated group (**Figure 4.5 A**). PQQ alone treatment had similar effect with no significant difference with EcN-5 treated group.

Moreover, we found significant increase in mRNA expression of PQQ target genes, PGC-1α (1.7 fold) and Tfam (1.5 fold) in aging rats treated with EcN-5 compared to untreated group (**Figure 4.5 B**). As a result, there was nearly 1.5 fold increase in hepatic mitochondrial content (Mit/Nuc DNA ratio) in these rats as compared to untreated ones (**Figure 4.5 C**). In this case also, no significant difference was observed between groups receiving PQQ alone and EcN-5 treatment.

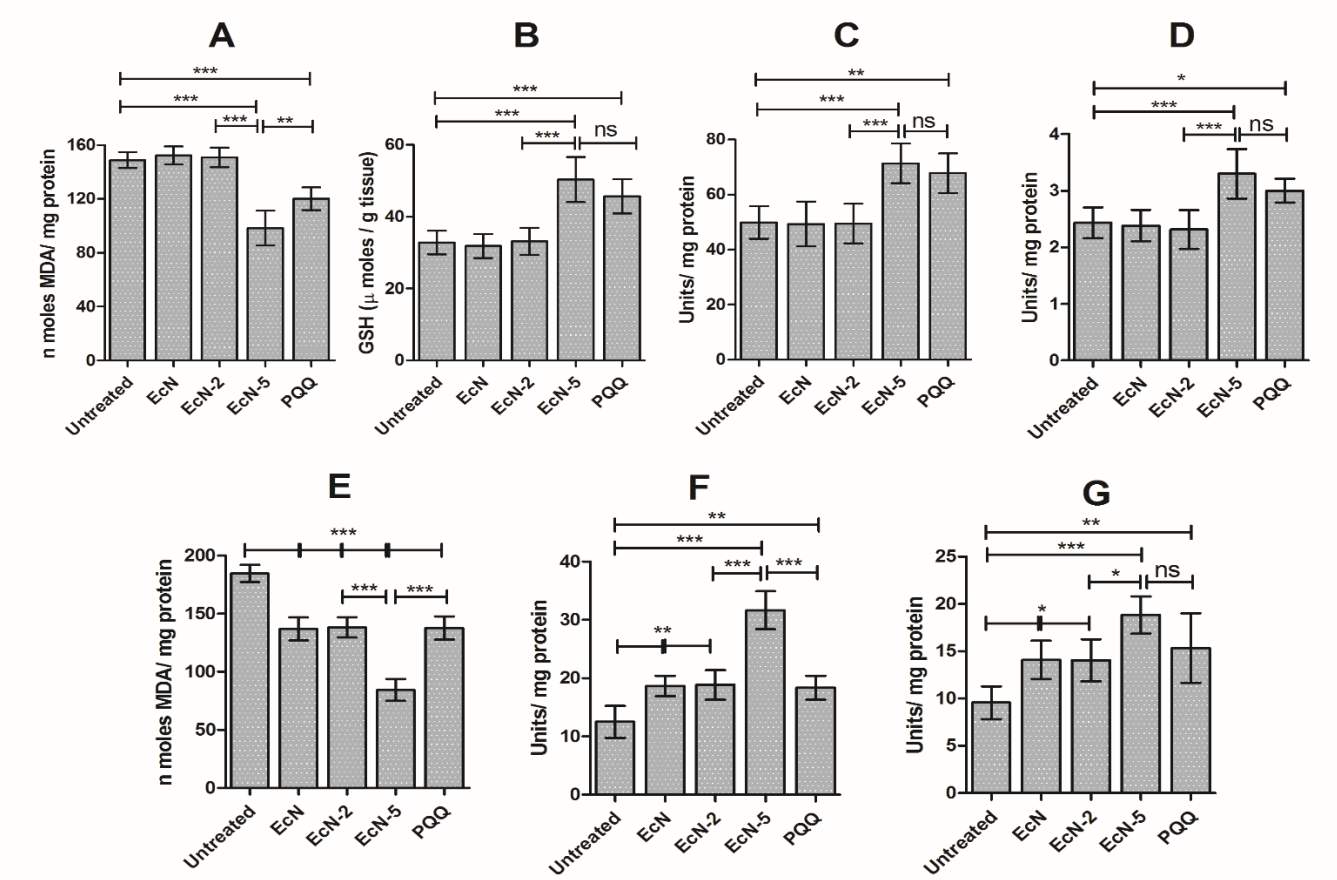

Figure 4.4 Antioxidant status in hepatic and colonic tissues of aging rats treated with PQQ producing EcN-5. 12 months rats were treated with respective treatments for 8 months. (A) hepatic lipid peroxidation (MDA levels), (B) hepatic GSH content, (C) hepatic CAT activity, (D) hepatic SOD activity, (E) colonic lipid peroxidation (MDA levels), (F) colonic CAT activity and (G) colonic SOD activity. *p<0.05, **p<0.01 and ***p<0.001 represent difference between different groups showed using horizontal bars.

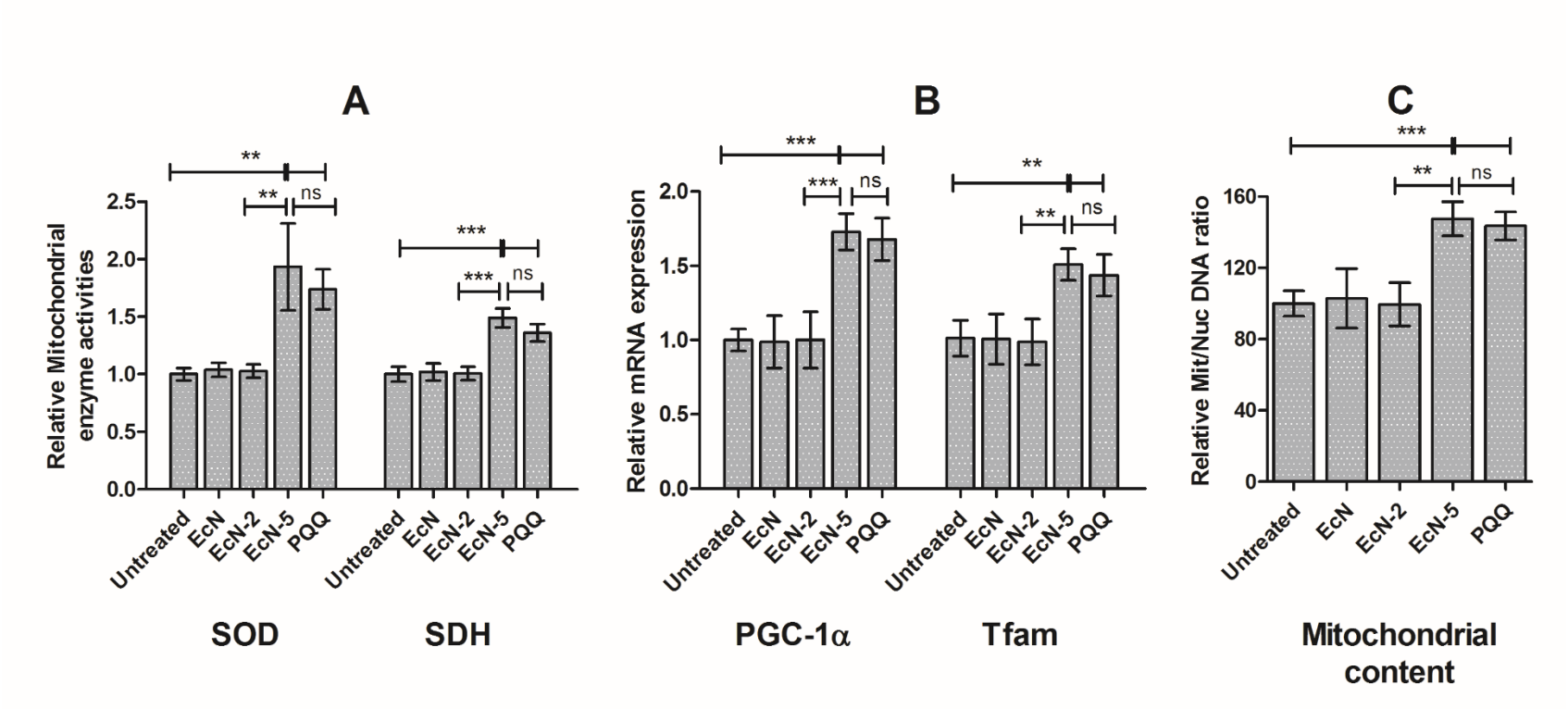

Figure 4.5 Mitochondrial metabolism, biogenesis and content in aging rats treated with PQQ producing EcN-5. Mit-SOD and Mit-SDH activities reflects mitochondrial metabolism (A). PGC-1α and Tfam mRNA expression reflects mitochondrial biogenesis (B). Relative Mitochondrial/Nuclear DNA ratio reflects mitochondrial content (C). **p<0.01 and ***p<0.001 represents difference between different groups showed using horizontal bars. All values are expressed as mean ± SD relative to control group (6 animals each group).

Lipid profile

Blood and hepatic lipid profile of all experimental animals is summarized in **Table 4.3**. Aging rats treated with EcN-5 showed significant reduction in blood **Triglycerides (16.5%)** and **total Cholesterol (23%)**. Hepatic Triglyceride and total Cholesterol were found to be reduced by **16% and 23% respectively** in rats treated with EcN-5 compared to untreated control. Moreover, rats treated with PQQ alone had similar blood and hepatic lipid profile as EcN-5 treated group.

Table 4.3 Plasma and hepatic lipid profile of aging rats after treated with EcN-5

Groups	Untreated	EcN	EcN-2	EcN-5	PQQ
Plasma TG	90.5 ± 8.2	88.2 ± 10.5	87.5 ± 12.2	75.5 ± 9.2 **	78.3 ± 10.8 *
Plasma CHO	105.2 ± 10.5	102.5 ± 12.3	98.8 ± 12.5	80.8 ± 9.0 **	82.2 ± 9.5 **
Hepatic TG	16.2 ± 2.1	16.8 ± 2.5	17.3 ± 1.8	13.5 ± 1.5 *	14.0 ± 1.7 *
Hepatic CHO	18.5 ± 2.6	17.2 ± 2.5	19.7 ± 3.1	14.2 ± 2.2 *	13.5 ± 2.8 *

All values are expressed as mean ± SD mg/dl of 6 animals each group.
Abbreviations: TG (triglycerides), CHO (total cholesterol).
Single asterisk indicates $p<0.05$ and two asterisks indicates $p<0.01$ relative to control group.

Hepatic lipid metabolizing gene expression

Hepatic mRNA expression of FAS and AcOx shows that aging animals treated with EcN-5 exhibit low expression of FAS (0.5 fold) and high expression of AcOx (2.25 fold) genes as compared to untreated control (**Figure 4.6 A-B**). PQQ alone treated group had similar expression level as EcN-5 treated.

Colonic Short Chain Fatty Acids (SCFAs) profile

SCFAs analysis of colonic content of experimental animals revealed significant increase in production of butyrate (1.8 fold), propionate (1.4 fold) and acetate (1.2 fold) in the rats treated with EcN-5 compared to untreated control (**Table 4.4**).

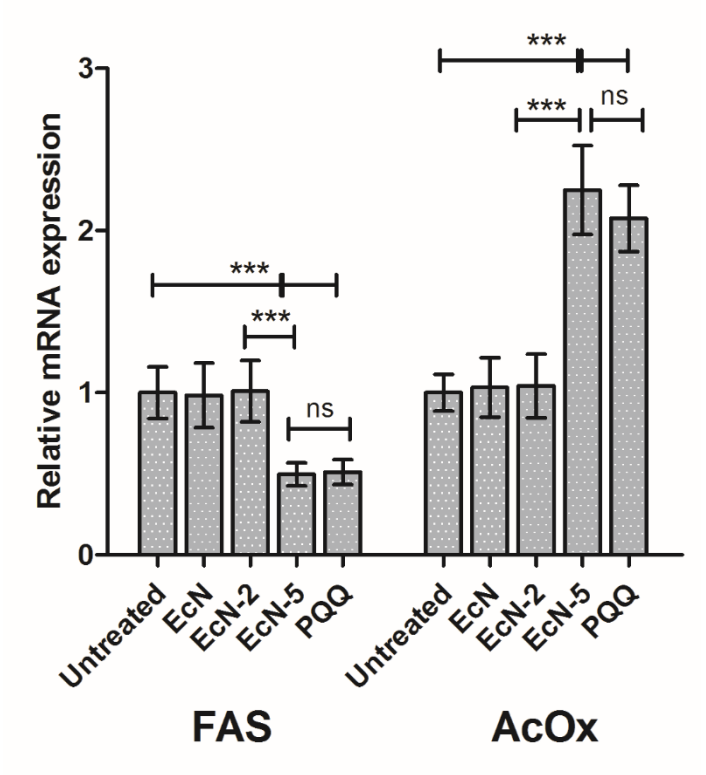

Figure 4.6. mRNA expression of lipid metabolizing genes. Fatty acid synthase (FAS) and Acyl Co-enzyme A oxidase (AcOx). ***p<0.001 represent difference between different groups showed using horizontal bars. All values are expressed mean ± SD relative to control group (6 animals each group).

Table 4.4 SCFAs concentration in colonic content of aging rats treated with EcN-5

Groups	Untreated	EcN	EcN-2	EcN-5	PQQ
Butyrate [1]	7.715 ± 1.33	7.275 ± 1.01	6.903 ± 0.68	14.92 ± 1.193 *** (1.8 fold)	10.968 ± 1.76
Propionate [1]	20.53 ± 2.32	19.551 ± 1.71	19.836 ± 1.99	29.956 ± 2.65 *** (1.4 fold)	23.815 ± 2.82
Acetate [1]	77.648 ± 3.76	78.603 ± 3.49	76.85 ± 3.94	91.492 ± 6.89 ** (1.2 fold)	85.157 ± 5.02

All values are expressed as mean ± SD of 6 animals each group.
[1] µ moles/ g wet colonic content. Fold changes are calculated with respect to untreated group. Three asterisks indicates p<0.001 relative to control group.

4.3.3 PQQ levels in fecal matter and liver if treated rats

Fecal matter and hepatic tissue were used for quantification of PQQ. EcN-5 treatment resulted in nearly 2.3 fold (fecal) and 5 fold (hepatic) increased in PQQ concentration compared to untreated control (**Table 4.5**). PQQ alone treatment also resulted in significant accumulation of PQQ in fecal matter and hepatic tissues as compared to untreated control.

Table 4.5 PQQ concentration in feces and liver of aging rats treated with PQQ secreting EcN-5

Groups	**Untreated**	**EcN**	**EcN-2**	**EcN-5**	**PQQ**
Feces [1]	0.735 ± 0.1	0.727 ± 0.114	0.703 ± 0.132	**1.715 ± 0.17** ***	**1.89 ± 0.21** ***
Liver [2]	25.04 ± 4.757	28.368 ± 5.015	30.095 ± 6.168	**124.925 ± 8.33** ***	**134.22 ± 10.23** ***

All values are expressed as mean ± SD of 6 animals each group. [1]nano moles/g fecal wet weight. [2]pico moles/ g tissue. Three asterisks indicates $p<0.001$ relative to control group.

4.3.4 Effect of PQQ on survival of *C. elegans* against heat shock and H_2O_2.

H_2O_2 when added in the media significantly reduces survival percentage of worms. But, PQQ when added on plates along with the worms, significantly increases survival percentage against H_2O_2 (10mM, 15mM & 20mM) **(Figure 4.7 A)**. Moreover, presence of PQQ in the medium also increased the thermo tolerance of worms at 35 ^{0}C. Worms treated with 5 µM PQQ survived for longer days as compared to untreated or treated with 2.5 µM PQQ **(Figure 4.7B)**.

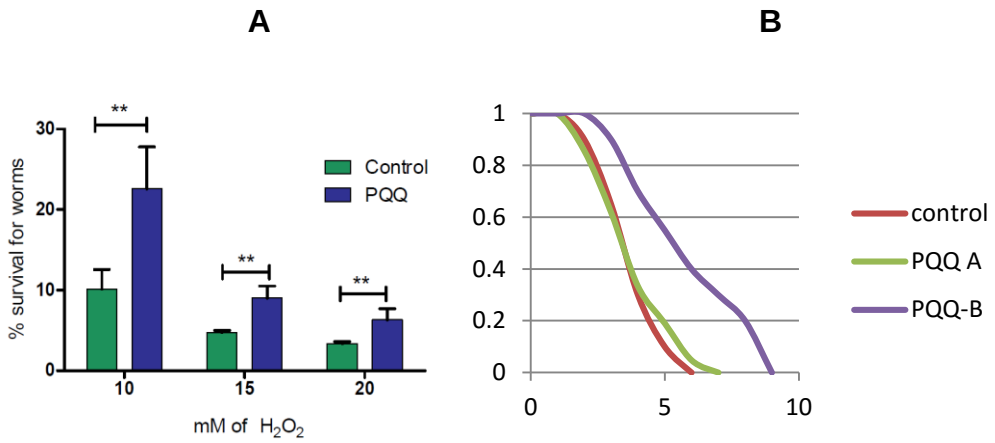

Figure 4.7 Effect of PQQ on *C. elegans* survival after H_2O_2 exposure (A) and heat (B). 5 µM PQQ was used against H_2O_2 (A). PQQ-A (2.5 µM) and PQQ-B (5 µM) concentration were used in thermotolerance assay (B).

4.4 Discussion

Aging, particularly in humans and other animals is highly dynamic phenomenon characterized by progressive deterioration of cellular machinery. Prolonged persistence of oxidative stress and inefficient antioxidant defence are considered as major factors influencing cellular aging. Mitochondrial metabolism is site for continuous production of free radicals during routine metabolism. These free radicals are by-products of electron transport during generation of ATP by respiration (Wallace, 2005). Many mitochondrial constituents such as mitochondrial genome and proteins are damaged in aging, which is consistence to the site of free radical generation. Moreover, studies have shown that individuals with genetic mitochondrial disease or rodent generating frequent mutations in mitochondrial genome, display pre-mature aging type phenotype (Kujoth et al., 2005; Trifunovic et al., 2004; 2005; Wallace, 2005). Therefore, it is considered that mitochondria play very important role in aging process. Calorie restriction (CR) is known to extend life span in rodents and other organisms probably by slowing carbohydrate use, respiration and the rate of ROS induced damage (Guarente, 2008). However, many studies have suggested that CR effects could be mediated by sirtuins in response to lower ATP/AMP ratio. Since, uncoupling mitochondrial DNA mutations, reduction in mitochondrial ROS production and mitochondrial damage are associated with increased life span, mitochondrial targeted molecules and antioxidants that limits mitochondrial ROS damage are more effective in countering oxidative damage associated with aging (Wallace, 2005; Dai et al., 2014).

PQQ, apart from strong antioxidant, also acts on cellular signalling pathways chiefly involved in mitochondrial biogenesis and cellular metabolism (Rucker, 2009). Evidences suggest that PQQ can permeate to subcellular organelles such as mitochondria (Sites et al., 2006). In the present study 3.7 Kb *pqqABCDE* gene cluster from *G. oxydans* 621H was used to reduce the plasmid load in contrast to our earlier reports of 13.3 Kb *pqq* gene cluster from *P. fluorescens* B16 (Pandey et al., 2014).

Vitreoscilla haemoglobin (VHb) encoded by *vgb* gene is known to induce growth under oxygen limiting condition, possesses peroxidase activity and prevents oxidative damage caused by carbon tetrachloride (Kumar et al., 2014). Presence of VHb improved colonization of probiotic *E. coli* CFR16 in the gut but did not exhibit any ameliorative effects in the present study. Since EcN strains used in the present study have *vgb* gene integrated in their genomes as a single copy, sufficient production of VHb may not have been achieved to exhibit the effect. This is in contrast to earlier reports using plasmid based expression system for *vgb* (Kumar et al., 2014).

Various compounds such as resveratrol, curcumin and carnosine have been used to minimize oxidative stress and cellular damage during aging in animals. Resveratrol acts on sirtuins exerting its effect on metabolism and thus acts similar to CR (Lagouge et al., 2006). However, it has very limited antioxidant potential. Curcumin is lipid soluble antioxidant and hence its antioxidant capacity is limited to membranes only. However, it is known to inhibit the promoters of inflammatory cytokines such as NF-kB, and thus reducing inflammation related to aging (Menon and Sudheer, 2007). Mitochondria are prime target of ROS which plays a central role in aging process. However, most of the natural compounds and molecules discussed earlier either limit in their antioxidant potential or limit in their effectiveness against mitochondrial damage or both. PQQ is a very novel class of antioxidant molecule with its presence in mitochondria allows it to counter mitochondrial specific oxidative stress. Since, earliest molecular theory of aging suggest role of mitochondrial generated ROS in the pathogenesis, and considering the outcome of previous experiment we hypothesized that PQQ producing EcN-5 could alleviate age associated systemic oxidative stress and hyperlipidemia in aging rodents. Aging rats treated with EcN 5 showed reduced oxidative stress in hepatic as well as colonic tissue by virtue of antioxidant property of PQQ. Moreover, increased hepatic mitochondrial content and metabolism are the result of novel function of PQQ in rejuvenating healthy mitochondria. This helps the cell to cope up with dysfunctional mitochondria and ATP crisis during pathogenesis (i.e. aging).

Integrity of intestinal barrier is very essential for maintaining proper functioning of the associated tissues (Shen et al., 2011). Intestinal barrier disruption, structural and functional impairments have been demonstrated in lower animals and mammals as well (Kirkwood, 2004; Rera et al., 2012). In the current study on aging rats, native EcN strains (EcN and EcN-2) lacking PQQ secreting capability could reduce colonic oxidative stress up to certain extent which is probably by virtue of their probiotic properties. Interestingly, incorporation of PQQ secreting capability enhances their ameliorative effect. Thus EcN-5 acts as continuous endogenous delivery system capable of secreting PQQ in the intestine. PQQ so produced is then absorbed and distributed to different parts of the body. High amount of PQQ found in fecal sample and liver of treated animals supports the earlier argument. High amount of SCFAs also play a major role in host metabolism especially related to lipid storage and obesity cells (Kondo et al., 2009; Al-Lahham et al., 2010; Arora et al., 2011; Kimura et al., 2013). Thus, the effect of EcN-5 on lipid accumulation and change in related hepatic gene expression could be attributed to the combined effect of PQQ and SCFAs both.

C. elegans is one of the preferred models for antiaging studies. PQQ was found to be protective against ROS and heat induced damages in worms. It increased their survival in these conditions. Further studies on murine models to understand the survival and longetivity could be important in this regard.