Review

Pyrroloquinoline-quinone and its versatile roles in biological processes

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Pyrroloquinoline-quinine (PQQ) was initially characterized as a redox cofactor for membrane-bound dehydrogenases in the bacterial system. Subsequently, PQQ was shown to be an antioxidant protecting the living cells from oxidative damage *in vivo* and the biomolecules from artificially produced reaction oxygen species *in vitro*. The presence of PQQ has been documented from different biological samples. It functions as a nutrient and vitamin for supporting the growth and protection of living cells under stress. Recently, the role of PQQ has also been shown as a bio-control agent for plant fungal pathogens, an inducer for proteins kinases involved in cellular differentiation of mammalian cells and as a redox sensor leading to development of biosensor. Recent reviews published on PQQ and enzymes requiring this cofactor have brought forth the case specific roles of PQQ. This review covers the comprehensive information on various aspects of PQQ known till date. These include the roles of PQQ in the regulation of cellular growth and differentiation in mammalian system, as a nutrient and vitamin in stress tolerance, in crop productivity through increasing the availability of insoluble phosphate and as a bio-control agent, and as a redox agent leading to the biosensor development. Most recent findings correlating the exceptionally high redox recycling ability of PQQ to its potential as anti-neurodegenerative, anticancer and pharmacological agents, and as a signalling molecule have been distinctly brought out. This review discusses different findings suggesting the versatility in PQQ functions and provides the most plausible intellectual basis to the ubiquitous roles of this compound in a large number of biological processes, as a nutrient and a perspective vitamin.

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1. Occurrence of PQQ in nature

Pyrroloquinoline-quinone (PQQ) was first identified in methylotrophic bacteria (Salisbury *et al.* 1979; Westerling *et al.* 1979) as a coenzyme for methanol dehydrogenase in 1979, and named as methoxatin. PQQ covalently interacts with different enzymes (McIntire 1994) and the proteins interacting with PQQ were originally termed as quinoproteins. Subsequently, it is found that topaquinone (TQ) and tryptophan tryptophenylquinone (TTQ) also act as coenzymes for many proteins. The proteins interacting with these quinones including PQQ and regulating their functions were also included in the category of quinoproteins. Although the majority of these proteins were bacterial dehydrogenases, a large number of other enzymes have also been found to require PQQ as a cofactor. Most notable ones are amine oxidase/dehydrogenase (McIntire 1994), Ser/Thr protein kinases from *Escherichia coli* (Khairnar *et al.* 2007), and *Deinococcus radiodurans* (Rajpurohit and Misra 2010), 2-aminoadipic 6-semialdehyde dehydrogenase from mammalian system and a signalling protein having AMP-binding and phosphopantetheine-binding domains and six PQQ-binding motifs (Wang *et al.* 2005). PQQ has been detected in a wide variety of foods and other sources (Kumazava *et al.* 1995; Mitchell *et al.* 1999). Quantitative analyses of PQQ by LC/MS/MS showed that free PQQ was present in almost all food samples, in the range of 0.19–7.02 ng per gm fresh weight (for solid foods) and per mL in liquid foods (Noji *et al.* 2007). Although, PQQ plays important roles in the growth and development of all organisms

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studied so far, its synthesis in higher organisms has not been shown. The major source of PQQ in these organisms is believed to be microbial sources. The amount of PQQ excreted from different microorganisms varies from 1 tg to 1 mg/mL, and is influenced by the composition of the growth medium (Duine 1991; Urakami *et al.* 1992).

The biochemical pathway of PQQ synthesis has been traced in *Methylobacterium extorquens* AM1 grown in medium supplemented with [^{4}C] tyrosine (Smidt *et al.* 1991; Junkefer *et al.* 1995). Genetics of PQQ synthesis has been studied in many bacteria including *Acinetobacter calcoaceticus* (Goosen *et al.* 1989), *Enterobacter intermedium* 60-2G (Kim *et al.* 2003), *Gluconobacter oxydans* (Hölscher and Görisch 2006), *Klebsiella pneumoniae* (Meulenberg *et al.* 1992), *M. extorquens* AM1 (Toyama *et al.* 1997) and *Pseudomonas fluorescens* CHA0 (Schnider *et al.* 1995). The number of genes involved in the synthesis of PQQ ranges from 4 in *A. calcoaceticus* to 6–7 genes in methylotrophs and *G. oxidans*. The majority of the bacteria making PQQ contain 6 or 7 genes (*pqqABCDEF/G*) in an operon. PQQ biosynthesis involves several enzymes encoded by these genes including *pqqE* that encodes a regulatory enzyme named as PQQ synthase (Goldstein *et al.* 2003). The *pqqA* encodes a small peptide of generally 24 amino acids bearing tyrosine and glutamate, which get fused and form a precursor for subsequent transformations into PQQ (Goosen *et al.* 1992). This molecule remains attached with a precursor peptide and is cleaved off at a later step in the biosynthetic process by other enzymes of the pathway. Although the *pqqA* gene product is redundant for the synthesis of PQQ in some of the bacteria, its availability seems to be a rate-determining step in PQQ biosynthesis.

Escherichia coli is deficient in PQQ synthase and, therefore, has been used as a host for cloning the PQQ synthase gene from *Ervinia harbicola* (Liu *et al.* 1992), *Pseudomonas cepasia* (Babu-Khan *et al.* 1995), *Rahnella aquatilis* (Kim *et al.* 1998), *E. intermedium* (Kim *et al.* 2003; Rodriguez and Fraga 1999) and *D. radiodurans* (Khairnar *et al.* 2003). Transgenic *E. coli* producing PQQ could solubilize insoluble rock phosphates into inorganic phosphorous (figure 1). Several DNA fragments have been identified from different bacteria that help in the synthesis of PQQ in *E. coli* and apparently share little or no sequence homology with the known PQQ synthase genes. This suggests the diversity in metabolic pathways associated with the synthesis of PQQ in different microorganisms. Since *E. coli* has been used for cloning of PQQ synthase genes from different bacteria using functional complementation of mineral phosphate solubilization (MPS) activity, the entire PQQ synthesis operon from *G. oxidans* has been expressed in *E. coli* and synthesis of PQQ has been demonstrated (Yang *et al.* 2010). Recently, the roles of PQQ have been shown in several other physiological processes. Readers are suggested to refer to the recent

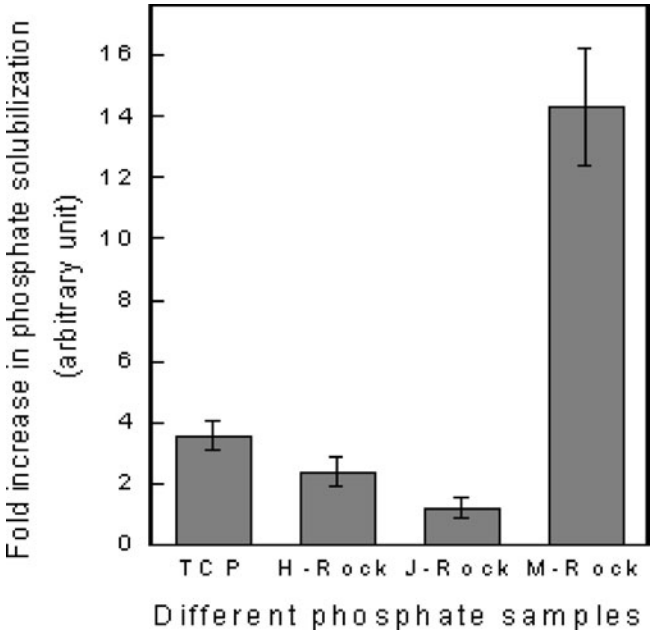

Figure 1. Functional complementation of mineral phosphate solubilization phenotype in transgenic *Escherichia coli.* The PQQ synthase of *D. radiodurans* was expressed in *E. coli*. Transgenic cells producing PQQ was compared with wild-type cells harbouring plasmid vector for release of inorganic phosphate from different samples containing insoluble phosphates such as tricalcium phosphates (TCP), and mineral rocks collected from Hirapur (H-Rock), Mussoorie (M-Rock) and Jhamarkatra (J-Rock) mines of India.

reviews published recently either on the enzymes requiring PQQ as a cofactor and/or on the specific role of this molecule (Willner *et al.* 2007; Hölscher *et al.* 2009; Rucker *et al.* 2009; Fetzner and Steiner 2010; Yakushi and Matsushita 2010). Here we reviewe the important findings that suggest the different roles of PQQ in the diversified cellular and molecular processes.

2. PQQ role in crop productivity

2.1 *Through phosphate solubilization*

Microbial biodiversity in soil plays a significant role in metabolism of complex molecules and production of antibiotics, secondary metabolites and other useful ingredients that contribtute to crop productivity. Photoautotrophic growth of plants, algae and photosynthetic bacteria requires several micro- and macronutrients. The availability of nitrogen and phosphorous as macronutrients is important for the growth of crop plants and agricultural productivity. These macronutrients are applied to the field in form of both easily digestible and bio-transformable chemical fertilizers. Efficient and environmentally regulated microbial processes help in

recycling of both these important macronutrients in nature (Rudolf and Kroneck 2005; Bhattacharjee *et al.* 2008; Cheng 2008). Molecular nitrogen is fixed to inorganic nitrogen through the highly regulated pathways in diazoptrophic bacteria, which then gets utilized in the synthesis of organic molecules through cellular nitrogen and carbon metabolism. Phosphorus the second most important macronutrient and can readily form the insoluble salts with different metal ions, rendering phosphorous easy availability for growth of the plants. Microbes present in the soil employ different strategies to make the unavailable forms of phosphates to the most readily useable form of phosphorous for plants. Like nitrogen fixation, the phosphate availability in soil has also been increased by improved microbial metabolism.

Several gram-negative bacteria are capable of producing organic acids by direct oxidation of aldehydes, which then get diffused in surroundings and help in the acidification of poorly soluble mineral phosphates (Goldstein 1986; Sashidhar and Podile 2010). Glucose dehydrogenase (GDH) requires PQQ as a redox cofactor for direct oxidation of glucose to gluconic acid, which then diffuses in the soundings of bacterial niche and helps in acidic solubilization of insoluble phosphates in soil. Both membrane-bound and soluble forms of GDH, in spite of having different substrate specificity, use PQQ as a cofactor. The role of PQQ as a redox coenzyme has been reported for several dehydrogenases, including methanol dehydrogenase, ethanol dehydrogenase and GDH (encoded by *gdh*) (Duine 1999; Matsushita *et al.* 2002; Stites *et al.* 2000). There are plant growth-promoting bacteria that use GDH-PQQ holoenzyme for solubilization of both inorganic and /or organic phosphates in soil (Han *et al.* 2008; Liu *et al.* 1992). In addition, the transformation of two rhizobacterial strains, *Burkholderia cepacia* IS-16 and a *Pseudomonas* sp. strain, with gene(s) encoding the enzymes of PQQ biosynthetic pathway, could enhance the MPS phenotype of these bacteria. *Azotobacter*, a free-living nitrogen fixer, known to increase the fertility of the soil, was engineered for PQQ synthesis, which could broaden its biofertilizer potential in crop producitivty (Sashidhar and Podile 2010). These studies suggest that microbes producing PQQ can increase the phosphate availability in soil for the growth and development of crop plants, which in turn increase crop productivity. The possibility of PQQ enhancing the growth of the crop plants also by the mechanisms that are beyond phosphate solubilization cannot be ruled out.

2.2 *As a bio-control agent and plant growth promoting factor*

The gram-negative bacterium *R. aquatilis* is ubiquitous and is characterized by its beneficial metabolism leading to mineral phosphate solubilization, antimicrobial activity, nitrogen fixation and plant disease suppression (Calvo *et al.* 2007). This bacterium produces PQQ and its MPS phenotype is contributed by mechanisms similar to other phosphate solubilizing microbes. The *R. aquatilis* HX2 has been used as a biocontrol agent for grapevine crown gall caused by *Agrobacterium vitis.* PQQ minus cells of this bacterium become ineffective in its bio-control activity. Expression of complete operon of PQQ synthesis restored the bio-control potential of this organism *in vivo*, suggesting the possible role of PQQ in controlling the host pathogen interaction (Guo *et al.* 2009). Several reports suggest that the GDH-PQQ holoenzyme is involved in production of antimicrobial substance in *P. fluorescens* (James and Gutterson 1986; Schnider *et al.* 1995; de Werra *et al.* 2009) and *E. intermedium* 60-2G (Han *et al.* 2008). *E. intermedium* 60-2G, a phosphate-solubilizing bacterium, has the ability to induce systemic resistance in plants against soft rot pathogen *Erwinia carotovora* and the mutation in *pqqA* and *pqqB* genes make these mutants lose their bio-control ability (figure 2). Interestingly, both *pqqA* and *pqqB* mutants of *E. intermedium* lost their bio-control ability for rice pathogen *Magnaporthe grisea* KI-409 and their ability to enhance the systemic resistance to the infection of fungal pathogens, suggesting that PQQ contributes in MPS, antifungal activity and in determination of induced systemic resistance of *E. intermedium* (Han *et al.* 2008). It is widely believed that PQQ promotes growth of both mammals and plants through

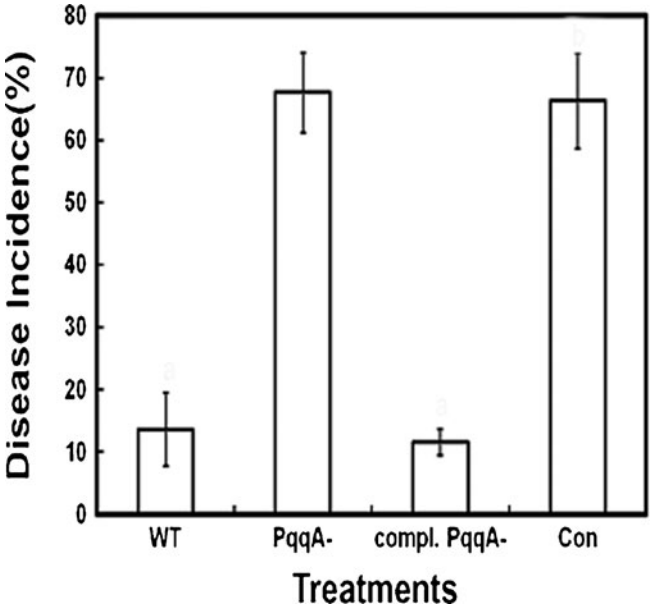

Figure 2. Role of PQQ in biocontrol of fungal infection in plants. Inactivating *pqqA* gene generated the PQQ-deficient mutant of Enterobacter intermedium 60-2G. The wild-type (WT) and PQQ minus cells (pqqA^{-}) alone, and mutant transformed with PqqA expressing on plasmid (compl pqqA^{-}) and plasmid vector control (Con), were compared for fungal disease incidence in plants (courtesy Professor YC Kim and colleagues; Han *et al.* 2008).

its antioxidant and MPS roles, respectively. However, recent studies on the role of PQQ in bio-control efficacy and as a plant growth-promoting factor have grown beyond its antioxidant and MPS roles. PQQ minus derivative of *P. fluorescens* B16, a plant growth-promoting rhizobacterium, lost its growth-promoting ability (Choi *et al.* 2008), which was functionally complemented by overexpression of all the proteins of the PQQ biosynthetic pathway *in trans*. Application of wild-type *P. fluorescens* B16 on tomato (*Solanum lycopersicum*) plants cultivated in a hydroponic culture system significantly increased the height, flower number, fruit number and total fruit weight, whereas none of the PQQ minus strains did that in spite of having the sufficient concentration of macronutrients including soluble phosphates. Recently, a bacterial isolate CMG 860 containing complete PQQ synthesis genes was shown to have plant growth promoting activity. The mutation in PQQ synthesis genes resulted in loss of plant growth promoting activity of CMG860 isolate (Ahmed and Shahab, 2010). Until recently, the beneficial effects of PQQ were conveniently explained on the basis of its antioxidant properties or ability to produce organic acids. Recently, it has been shown that supplementation of 5 to 1000 nM synthetic PQQ in plants nutrients could significantly increase the fresh weight of cucumber (*Cucumis sativus*) seedlings (Choi *et al.* 2008) (figure 3), confirming that PQQ is a plant growth-promoting factor by yet-unexplored mechanisms. These findings indicated the role of PQQ in crop productivity, which seems in addition to its roles as an antioxidant and in the solubilization of insoluble phosphates.

3. PQQ role in health sciences

3.1 *In oxidative damage tolerance and redox control*

Many naturally occurring quinones have been used as drugs to protect cells against oxidative stress (Halliwell and Gutteridge 1999) *in vivo*. These quinones react with reactive oxygen species (ROS) and produce oxidation products, which could form adduct with glutathione (GSH), resulting in depletion of free GSH. This results in reduced oxidative stress tolerance and, therefore, the oxidation of biomolecules including proteins and manifestation of the oxidative killing of cells (Boots *et al.* 2003, 2007). The molecular mechanisms of quinone cytotoxicity have been extensively reviewed (Bolton *et al.* 2000; Monks and Jones 2002). Like other quinone-based antioxidants, PQQ also works in concentration-dependent manner. Up to 10 µM, it works predominantly as antioxidant, while beyond 50 µM it acts as pro-oxidant. Both the antioxidant and pro-oxidant nature of PQQ have functional significance in biology (He *et al.* 2003). Being highly electrophilic, this compound reacts with many substances through its 5 positions. It forms stable

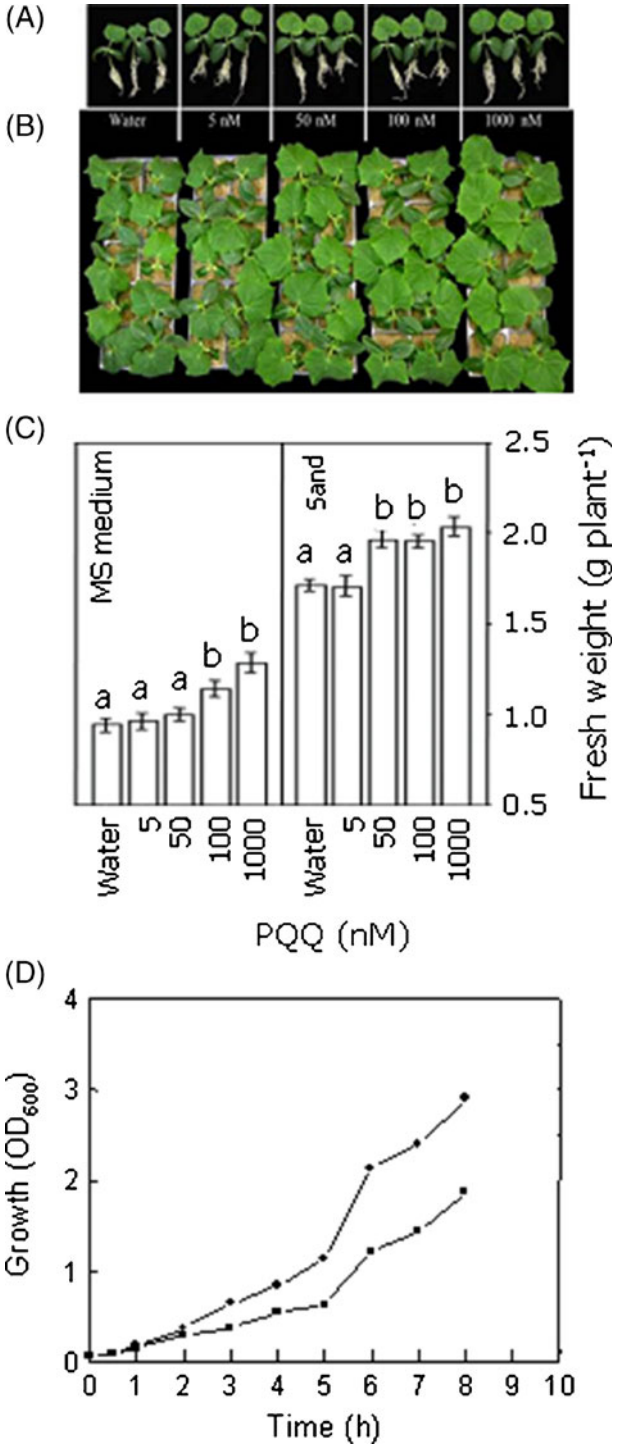

Figure 3. PQQ effect on growth in bacteria and plants. Plants were grown in both minimal medium (MS) (**A**) and soil (sand) (**B**), supplemented with different concentration of PQQ. The growth of these plants were measured as height and compared with control plants grown without PQQ (**C**) (courtesy Professor Ingyu Hwang and colleagues; Choi *et al.* 2008). Transgenic *E. coli* producing PQQ was compared with *E. coli* transformed with plasmid vector as control and growth was measured as optical density at 600 nm (**D**).

adducts with carbonyl reagents like hydrazine, phenylhydrazines, hydroxylamine, and semicarbazide, and the complexes with acetone, aminoguanidine, urea, o-phenylene diamine, sulfite and malononitrile. PQQ reacts with ammonia and produces iminoquinone, which has a *Kd* of 41 mM, and this adduct has pKa values of 9.1 and 11.86. These characteristics provide PQQ the ability to oxidize the redox modulatory site of *N*-methyl-D-aspartic acid (NMDA) receptors, thus conferring protection against NMDA- or glutamate-mediated cell injury in cultured neurons (Aizenman *et al.* 1992). Recently, our findings have suggested that PQQ has a role in selective killing of U937 human promonocytic cells largely by changing the intracellular redox status (Shankar *et al.* 2010). PQQ induces apoptosis in U937 cells, which increased 2- to 5-fold in the presence of NAC or GSH, suggesting the possible role of PQQ-mediated cytotoxicity beyond its redox regulation. Depletion of cellular glutathione, on the other hand, amplified the cellular toxicity of PQQ by several folds and also switched the mode of PQQ-induced cell death from apoptosis to necrosis (Shankar *et al.* 2010). This might indicate that PQQ exerts necrotic cell death by its oxidative effect but increased apoptosis in cells protected from oxidative stress, which appears to be due to its effect beyond pro-oxidant. The most promising potential application of PQQ has been in neuroprotection, which could be implicated either the functioning of PQQ as a redox control and response to oxidative stress or as a signalling molecule helping in the growth of neurons. These finding, nevertheless, suggested that PQQ regulates redox status of the cells, it also regulate the cellular response of cytotoxicity by cell signalling.

Involvement of PQQ in modulation of oxidative stress response of living cells has been observed in both bacteria and mammalian cells. This property of PQQ is of greater significance in health science. The antioxidant nature has been implicated in the several beneficial effects seen in mammalian system fed with PQQ-supplemented diet. PQQ has been known as an essential nutrient, which also protects neurological cells by suppressing peroxynitrile formation (Zhang and Rosenberg 2002) and blocks SIN-1-evoked ATP depletion and nitration of bovine serum albumin by scavenging superoxide radical. The SIN-1 and peroxynitrile pathways of oxidative stress modulation have been correlated with oxidative-stress-induced signal transduction pathway. Further, PQQ has been shown as a neuroprotectant. It prevents the neurotoxin 6-hydroxydopamine (6-OHDA)-induced cell death and DNA fragmentation in SH-SY5Y cells. Since the 6-hydroxydopamine (6-OHDA) generates ROS, the protection from the cytotoxic effect of 6-OHDA by PQQ suggested that PQQ functions as ROS scavenger, especially superoxide (Hara *et al.* 2007). It has been shown that *E. coli* cells producing PQQ could tolerate the mixed ROS produced from the photodynamic effect of Rose Bengal (5 μg/mL), several fold higher than the control (figure 4) (Khairnar *et al.* 2003). These cells also showed ~4-fold higher protection of proteins from γ-radiation-induced DNA damage as compared with non-engineered cells. This suggested a role of PQQ in protection of bacterial cells against oxidative damage (Khairnar *et al.* 2003). PQQ could also neutralize the artificially produced ROS such as superoxide, hydroxyl and oxygen free radicals and produces non-reactive adducts with ROS *in solution* (Misra *et al.* 2004). In an independent study, the phosphate-solubilizing bacteria isolated from phosphate-deficient soils were checked for oxidative stress tolerance. The bacteria producing PQQ showed higher tolerance to hydrogen peroxide and γ radiation effect (Shrivastava *et al.* 2010). This suggested that PQQ controls the oxidative stress response of the cells by acting as antioxidant/pro-oxidant; its role in stress response regulation beyond these characteristics would be worth appreciating. It is noteworthy that PQQ responses to oxidative stress in prokaryotes and eukaryotes are widely different, indicating the mechanistic difference of PQQ functions in bacteria and higher organisms.

3.2 *Clinical implications of PQQ*

The effect of PQQ on induction of nerve cells was observed in sciatic-nerve-deficit model created in rats (Liu *et al.* 2005). They observed that the PQQ-treated experimental samples produced more mature and high-density regenerated nerves cells, suggesting that PQQ is a potent enhancer for the regeneration of peripheral nerves. The clinical uses of PQQ have also been shown in stroke therapy using rats as model system and demonstrated that PQQ alone could enhance mitochondrial respiratory ratios in ischemic and non-ischaemic myocardium (Zhu *et al.* 2006). PQQ showed better protection of mitochondria from ischaemia/reperfusion oxidative damage as compared with the most effective drug metoprolol. PQQ has been demonstrated for its role in modulation of mitochondrial quantity and function in mice (Stites *et al.* 2006). They have shown that PQQ stimulates mitochondrial complex 1 activity *in vitro* and counters the effect of mitochondrial complex 1 inhibitor diphenylene iodonium action *in vivo*. PQQ has been shown to play a role as an antioxidant in neuronal cells and to prevent neuronal cell death in a rodent stroke model. The levels of expression and oxidation status of DJ-1, a causative gene product for a familial form of Parkinson's disease, were also reduced in primary cultured SHSY-5Y neurons cells when treated with 6-hydroxydopamine (6-OHDA) or H_2O_2 in the presence of PQQ. Thus, the neuroprotective effects of PQQ on oxidative-stress-induced neuronal death could be speculated (Nunome *et al.* 2008). PQQ protection from methyl-mercury-induced neurotoxicity in PC12 cells and NMDA-receptor-mediated neurotoxicity to the spinal cord (Zhang *et al.* 2006;

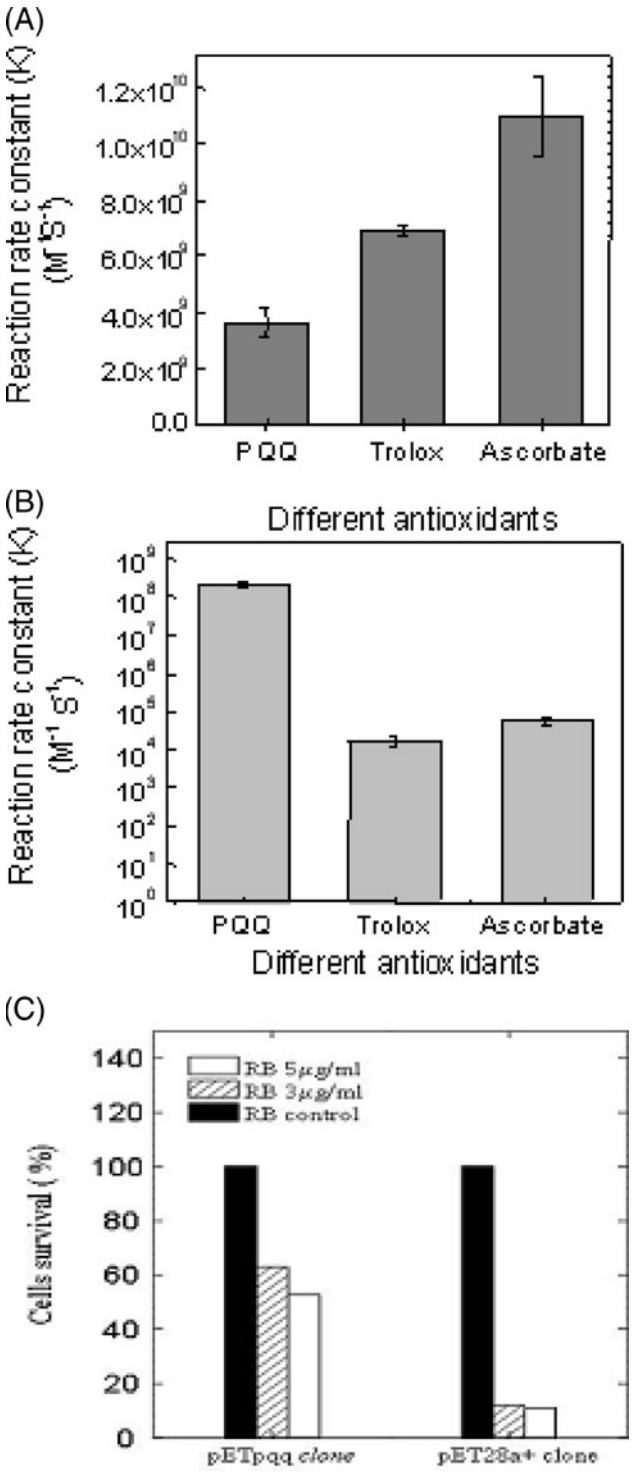

Figure 4. Role of PQQ as an antioxidant and in oxidative stress response in bacteria. The reactivity of PQQ with artificially produced hydroxyl (**A**) and superoxide (**B**) free radicals were compared with known antioxidants like ascorbate and 'Trolox'. *Escherichia coli* making PQQ were checked for mixed reactive oxygen species produced at different concentrations of Rose Bengal (**C**) under constant light illumination.

Zhang *et al.* 2009) and on neurotoxicity of aggregated beta-amyloid (Abeta), a critical cause in the pathogenesis of Alzheimer's disease (AD) (Hara *et al.* 2007), has been demonstrated. A few notable effects of Abeta, such as the decrease in Bax/Bcl-2 ratio, and suppression of caspase-3 cleavage, were markedly reversed by PQQ. The effect of PQQ on the amyloid formation and inhibition of cytotoxicity of truncated alpha-synuclein provided the possible mechanism of PQQ action in protection of neuronal cells from Abeta toxicity. It has been shown that the aggregation and cytotoxicity of C-truncated alpha-synuclein 119 and alpha-synuclein 133, which have been found in both the normal and the pathogenic brains, were inhibited by PQQ. PQQ dramatically inhibits the fibril formation of C-terminal truncated alpha-synuclein 110, 119 and 133 as well as the mixtures of full-length alpha-synuclein with these truncated variants and thereby decreases the cytotoxicity of truncated alpha-synuclein (Kim *et al.* 2010a, b). They showed that PQQ at 10 mg/kg infused at the initiation, or 3 h after the initiation of reversible middle cerebral artery occlusion (rMCAo), was effective in reducing cerebral infarct volumes measured 72 h later. These results indicated that PQQ could protect neuronal cells against beta-amyloid-induced neurotoxicity. Although the mechanism of PQQ action in inhibition of fibril formation of C-terminal truncated alpha-synuclein is not clear, these findings suggested the pharmacological usefulness of PQQ and opened a possibility of it becoming a potent anti-neurodegenerative compound in the treatment of neurodegenerative diseases.

4. PQQ roles in stress response and signal transduction

Recently it has been shown that PQQ could activate the Ras signalling pathways in NIH3T3 mouse fibroblasts (Kumazawa *et al.* 2007) and could induce the differential phosphorylation of signalling proteins. On the one hand, PQQ treatment causes quick activation of ERK and PKC-epsilon and increases the phosphorylation of Rb and c-Jun. On the other, its presence down-regulates the expression levels of growth inhibitory molecules like IkappaB and p27. PQQ also counteracts the effect of soluble NSF attachment proteins (SNAP) and effect of growth inhibitors, and activates Ras pathway kinases, which lead to a dynamic shift in G0/G1 population to S and G2/M population. This suggested the role of PQQ in cell proliferation through Ras-mediated signalling pathways. Subsequently, the effect of PQQ on Schwann cell growth and AKT signalling pathways were evaluated (He *et al.* 2010). It was found that PQQ could affect the morphology of Schwann cells and activates AKT, indicating that the PI3K/Akt signalling pathway might be involved in Schwann cells proliferation and may be regulated by PQQ. PQQ effects on activation of both signalling protein kinases and oncogenic phosphoproteins, and the

regulation of gene expression in the mammalian system, have been documented (Rucker *et al.* 2009). Cellular differentiation in the mammalian system and increased apoptosis in U937 tumour cells (Shankar *et al.* 2010) are the other examples controlled by different signalling processes where PQQ effects have been observed. PQQ's involvement as a bio-control agent (Bashan and de-Bashan 2002; Han *et al.* 2008), a plant growth stimulation factor (Choi *et al.* 2008) and in the nodulation efficiency of *Sinorhizobium meliloti* by modulating the plant-bacterium interactions (Bernardelli *et al.* 2008), are other examples in heterologous system, which is associated with host pathogen response mechanisms. Therefore, the involvement of PQQ in signal transduction mechanisms in various biological processes is being increasingly appreciated.

Database search of proteins containing PQQ binding motifs showed that a large number of protein kinases from different organisms have multiple PQQ-binding motifs (figure 5). Among these, a nutritional stress responsive protein kinase PknD of *Mycobacterium* also has multiple PQQ-binding motifs (*http://smart.embl-heidelberg.de/smart*). In bacteria, the role of PQQ as an inducer of a periplasmic protein kinase that has a role in cell membrane biogenesis (Wu *et al.* 2005; Charlson *et al.* 2006), invasiveness of pathogenic *E. coli* (Rolhion *et al.* 2005**)** and DSB repair (Khairnar *et al.* 2007) have been reported in *E. coli*. Further, it has been demonstrated that *D. radiodurans*, an extremely radiation-resistant bacterium, lacking PQQ, loses its resistance to various DNA damaging agents (Rajpurohit *et al.* 2008). Here we noticed that cells lacking this cofactor had a different phosphoprotein profile than wild-type cells. Subsequently, it was found that bacterial proteins having multiple interacting sites for PQQ and eukaryotic type Ser/Thr kinase domain were stimulated by PQQ and had an important role in radiation resistance and DSB repair (Rajpurohit and Misra 2010) (figure 6). These findings are increasingly supportive of the role of PQQ in signal transduction mechanisms both in eukaryotes and prokaryotes.

5. Development of bioelectronics around PQQ

PQQ works as a redox cofactor for GDH for the direct oxidation of glucose to gluconic acid. During this process, PQQ develops a measurable redox potential, which has been exploited in the development of biosensors for measuring the glucose levels *in solution*. A PQQ-mediated glucose-oxidation-based bio-fuel cell with electrochemically switchable and tunable power output has been developed with certain modifications (Katz and Willner 2003; Katz

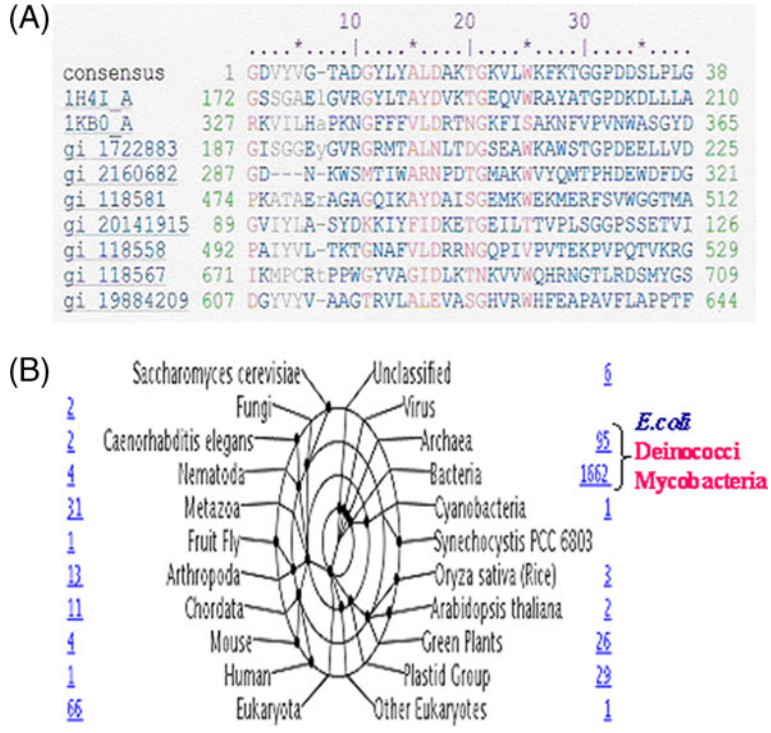

Figure 5. Distribution of PQQ binding proteins in different organisms. The consensus PQQ binding motifs deduced from quinoprotein dehydrogenases (**A**) were BLAST-searched in different proteins sequences submitted to public databases. The numbers of proteins showing PQQ binding motifs in different organisms are shown as underlined numbers. Three important bacterial species (*E. coli*, *Deinococci* and *Mycobacteria)* also showed many proteins with PQQ binding motifs (**B**) (*http://smart.embl-heidelberg.de/smart*).

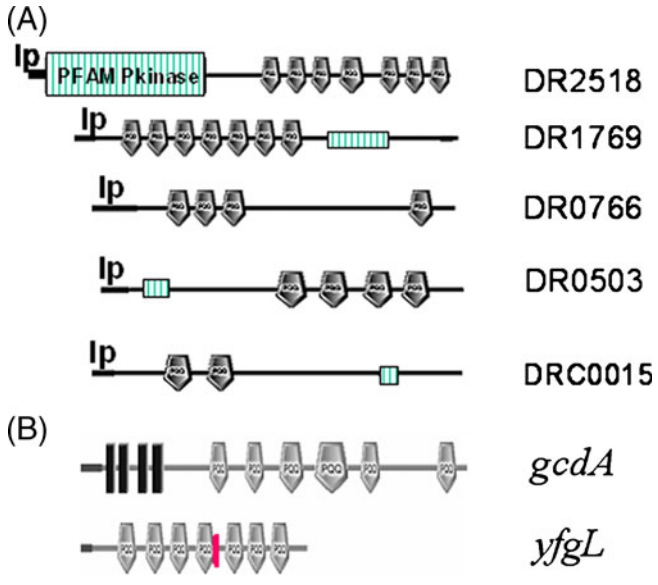

Figure 6. Different open reading frames in *Deinococcus radiodurans* R1 (**A**) and *Escherichia coli* K-12 (**B**) containing multiple PQQ binding motifs also in two well-characterized protein kinases like DR2518 (Rajpurohit and Misra 2010) and YfgL (Khairnar *et al.* 2007) (*http://smart.embl-heidelberg.de/smart*).

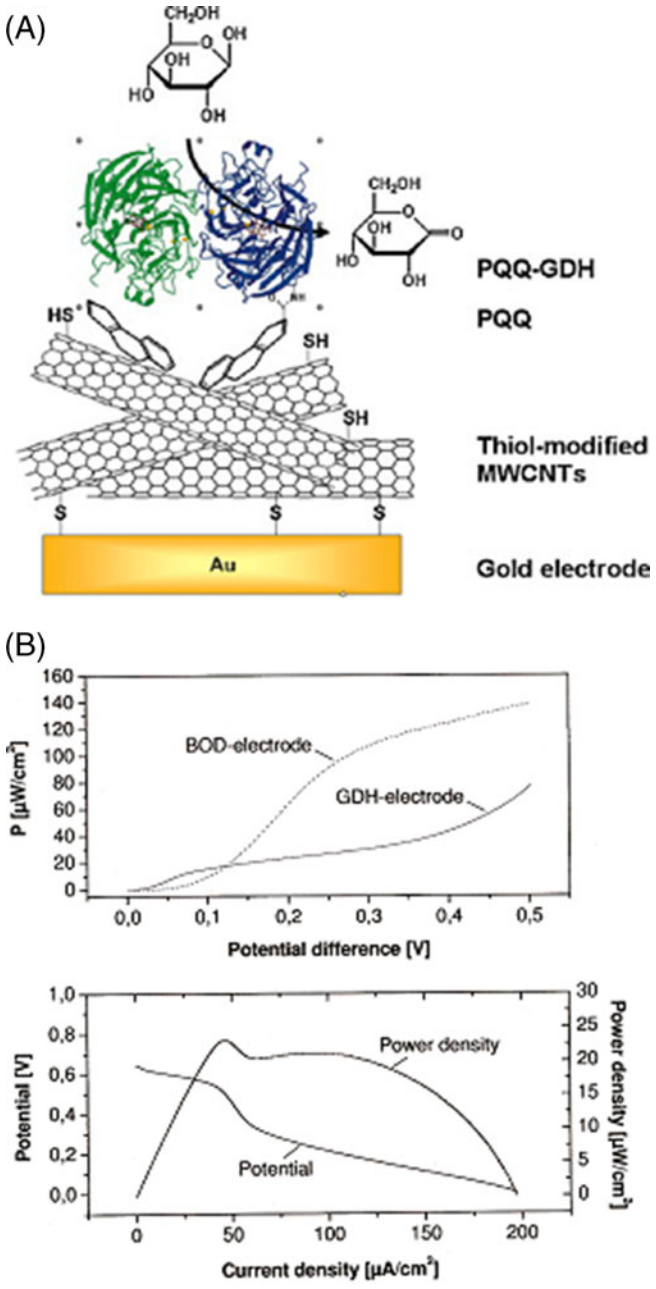

Figure 7. PQQ use in the development of biosensor. The redox characteristic of PQQ has been exploited for the development of glucose-based biosensor. Schematic representation of the development of membrane-free bio-fuel cell based on multiwalled carbon nanotubes (MWCNT)-modified gold anode coupled with PQQ-GDH (**A**) and performance testing of BOD and GDH bio-cell potential and power generation (**B**). (Courtesy to Professor F Lisdat and colleagues; Tanne *et al.* 2010).

et al. 2005; Yuhashi *et al.* 2005). Incorporating nanotubes into this technology has allowed an increase in the current density of ethanol/air bio-fuel cell by up to 14.5-fold and an increased power density by up to 18.0-fold. The use of PQQ in direct oxidation of other compounds including lactate (Treu and Minteer 2008) has widened its application in development of biosensors and bio-fuel cells. Thiol-modified multiwalled carbon nanotubes (MWCNT) were developed with the coating of a layer of PQQ-GDH and PQQ-LDH and used for detection of glucose and lactate levels. This technology has been combined with a bilirubin oxidase (BOD)-coupled MWCNT-modified electrode, and a membrane-free bio-fuel cell with an open cell potential of 600 mV and a power density in the range of 23 μW/cm has been made (Tanne *et al.* 2010) (figure 7).PQQ-dependent soluble GDH-based carbon paste electrodes have been developed for glucose monitoring in oxygen-deficient media and for testing yeast fermentation capacity in an oxygen-independent manner (Kurtinaitienė *et al.* 2010). The enhancement of PQQ-dependent GDH activity by artificial electron acceptors has been demonstrated (Kulys *et al.* 2010). Glucose oxidase was immobilized to the PQQ cross-linked modified amphiphilic phospholipid polymer (PMBN), which produces electrochemical oxidation of glucose on the polymer electrode. This suggested the possibility of PMBN use in enzyme electrode for bioelectronics (Yu *et al.* 2010). Carbon nanomaterial supports have been employed in conjunction with heme-containing PQQ-dependent alcohol dehydrogenase (PQQ-ADH) and aldehyde dehydrogenase (PQQ-AldDH) enzymes. This functions as oxidation catalysts for producing stable, high-surface-area

catalyst supports for the bioelectrocatalysis of ethanol in bio-fuel cells (Willner *et al.* 2007; Treu *et al.* 2010). PQQ-apoglucose dehydrogenase (apo-GDH), loaded into poly (methyl methacrylate) (PMMA) nanospheres, when incubated in 40% acetonitrile, releases PQQ-GDH from the nanospheres and helps in glucose oxidation. The electron released during redox reaction is captured by another redox dye reagent, e.g. 2,6-dichloroindolphenol (DCPIP), and decreases its absorption. This allows the loading of an excess apoenzyme, which increases the detection capability with the increase in release of encapsulated PQQ from the surface-bound nanospheres (Shen and Meyerhoff 2009). The findings reviewed the use of PQQ in development of biosensor and bio-fuel cells, strongly support the possible use of this compound in the development of advanced bioelectronics system with an advanced nanomaterial technologies. The possibility of exploiting the redox nature of PQQ for development of technology in biological hydrogen production might be proposed.

6. PQQ roles in gene expression and protein functions

PQQ has been classified as an important nutrient (Killgore *et al.* 1989) and its effects on growth and stress tolerance of organisms have been studied by either supplementing diet with PQQ or producing this chemical inside the cells, at least in bacteria. The presence of PQQ even at pg/mL level in the culture medium stimulated bacterial growth by reducing the lag time, suggesting an important role of PQQ in the initiation of cell division (Ameyama *et al.* 1988). Balb/c mice fed chemically defined amino-acid-based diets supplemented with 6 μg PQQ per kg diet showed an improvement in its reproductive performance, growth, modulation of indices of neonatal extracellular matrix production and maturation in mice (Steinberg *et al.* 1994; Steinberg *et al.* 2003). Molecular mechanisms underlying roles of PQQ in growth and stress tolerance are partly known. The effect of PQQ has been demonstrated on both proteins synthesis at transcription level and post-translation regulation of protein functions by phosphorylation. PQQ-mediated inhibition of melanin synthesis in cultured melanoma cells was the first evidence that showed the direct role of PQQ in gene expression (Sato and Toriyama 2009). Here they showed that the tyrosinase and TRP-2 genes expression was differentially regulated in the presence of PQQ. Intraperitoneal administration of PQQ represses the synthesis of inducible nitric oxide synthase (iNOS) mRNA at the transcription level, in the injury site, and thereby effectively promotes the functional recovery of SCI rats (Hirakawa *et al.* 2009). Recently, PQQ effect on global gene expression has been studied both in the mammalian system and in bacteria. It is shown that the presence of exogenously supplemented PQQ in feed influences the expression pattern of several genes in rats (Tchaparian *et al.* 2010). It was shown that PQQ deficiency affects the expression pattern of 438 genes ($P<0.01$), which gets reversed when PQQ was supplemented in the diet. The genes most affected were responsible for cellular stress, mitochondriogenesis, cell signalling and MAP kinase pathways, and transport of metabolites. PQQ stimulates mitochondrial biogenesis through the

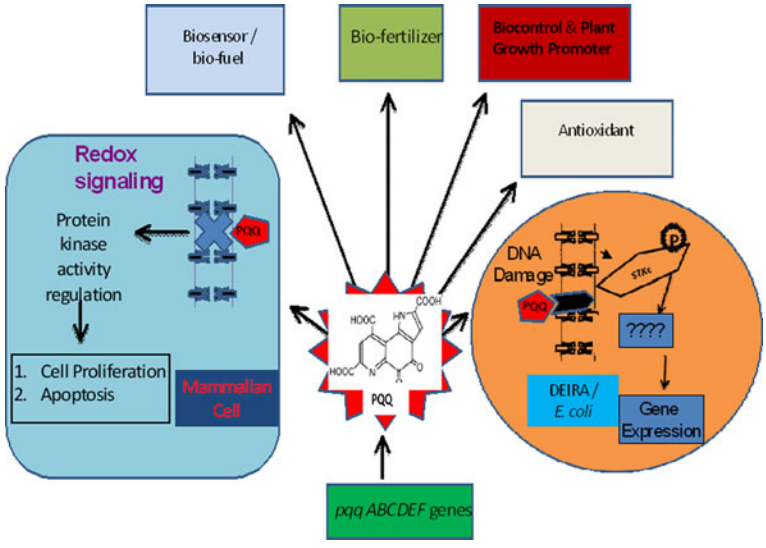

Figure 8. PQQ functions as a glance. Biochemical pathway of PQQ synthesis involves *pqqABCDEF* genes in bacteria. PQQ has roles in various processes, for example, as an inducer of protein kinases from both eukaryotes (redox signalling) and prokaryotes (DNA damage), bio-analytical devices (biosensor/ bio-fuel) solublization of insoluble phosphates (bio-fertilizer), crop productivity (bio-control and plant growth promoter) and protection from oxidative stress (antioxidant) have been profusely demonstrated.

activation of cAMP response element-binding protein (CREB) and peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1alpha) (Chowanadisai *et al.* 2010). Activation of the PGC-1 alpha pathway has been suggested through the PQQ-mediated nuclear respiratory factor activation (NRF-1 and NRF-2) and mRNA synthesis of Tfam, TFB1M, and TFB2M proteins. PQQ stimulates nerve growth factor (NGF) synthesis and secretion in Schwann cells. PQQ inhibition of amyloid fibril formation by inhibiting the synthesis of amyloid proteins, amyloid beta and mouse prion proteins has been reported (Kim *et al.* 2010a, b). In bacteria, the effect of PQQ on activity of enzymes has been known for a long time. PQQ is required as a redox cofactor for the activity of several membrane as well as soluble dehydrogeases in both prokaryotes and eukaryotes. Recently, it has been shown that PQQ could stimulate the activity of purified recombinant periplasmic protein kinase in *E. coli* (Khairnar et al 2007) and recombinant Ser/Thr protein kinase from *D. radiodurans* (Rajpurohit and Misra 2010). *Deinococcus* cells lacking PQQ and PQQ-stimulated eukaryotic type Ser/Thr protein kinase (STPK) separately showed an increased synthesis of 75 transcripts, while 200 genes were down-regulated by 1.5- to 15-fold ($P \leq 0.05$). Some of the important proteins showing changes in expression levels in these mutants were putative stress response proteins, proteins involved in energy metabolism, synthesis of biomolecules and DNA metabolism. Transcriptome data of PQQ-deficient mutants of *D. radiodurans* R1 have been submitted to Gene Expression Omnibus database (accession numbers GSE17722, GSM442538 and GSM442540). These findings clearly suggest the role of PQQ at the activity modulation of various enzymes and at the transcription level by still unknown mechanisms.

7. Conclusion

PQQ has been shown to be a ubiquitous molecule that affects numerous physiological and biochemical processes and has proved to be beneficial for growth and stress tolerance in both bacteria and higher organisms. Molecular mechanisms underlying the versatile nature of PQQ might not be merely accounted to its antioxidant property and/or to its role in solubilizing insoluble phosphates for easy availability for the growth of plants. Therefore, PQQ could be suggested as having roles in a process that might be as ubiquitous as its presence across the living system. One such mechanism suggested could be a signal transduction where PQQ could act as an inducer of protein kinases, which directly or indirectly regulate the functions of numerous proteins and also gene expression in response to both biotic and abiotic stresses. Since several protein kinases, neuronal growth factors, eIF2alpha/beta, etc., from bacteria, plants, animals and mammalian systems, contain PQQ-binding motifs and could be stimulated by PQQ, it seems quite convincing that PQQ has a role in different types of signalling mechanisms that regulate various physiological and molecular processes (figure 8). The redox recycling property of PQQ makes it a good candidate for redox controlled signalling in living cells and also for the development of bioelectronics for industrial applications. Although, its pro-oxidant as well as antioxidant properties has been exploited in health sciences, reducing its pro-oxidant characteristics without compromising the anti-oxidant and signalling properties would be a challenging task but the most desired development the future may like to witness. When that is achieved, this compound might act as an efficient anti-neurodegenerative, anticancer, and pharmacological agent. This review has brought forth the cognizance of the recent research emphasizing the usefulness of PQQ in agricultural, medical and industrial applications.

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