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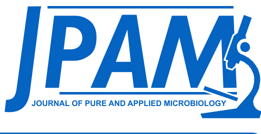

RESEARCH ARTICLE OPEN ACCESS

Molecular Cloning, Expression, and Function of *Synechocystis* PCC6803 Type II Peroxiredoxin (*sll1621*) Gene in *Escherichia coli* Cells under Salinity Stress Conditions

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Abstract

Microorganism's cycle exposure to reactive oxidants from internal metabolism and abiotic stress conditions, e.g. oxidative stress, salinity, drought, low temperature, high temperature, and high light. *Synechocystis* PCC6803 genomes typically encode different types of antioxidant scavenging enzymes including Peroxiredoxins (Prxs). There are five genes similar to Prxs were found inthe *Synechocystis* PCC 6803 genome. Based on sequence homology analysis of *Synechocystis* PCC 6803*sll1621* gene, it is categorized into type II Prx (PrxII). The presumed amino acid sequence of *Synechocystis* PCC6803 PrxII protein exhibited identity about 44%-99% to other PrxII proteins from human, plants, algae, and other different cyanobacterial cells. In the last decade, the genetically controllable model organism *E. coli* has been introduced as a viable biotechnological model for genetically modified microorganisms. The *Synechocystis* PCC6803 *sll1621* gene was overexpressed in pTYB21 expression vector and the resulting was named as *pTYB21/sll1621*. The *pTYB21/sll1621* was overexpressed in *Escherichia coli* BL21 (DE3) host cell. The overexpressed protein of PrxII gave the recombinant *E. coli* cells the ability to survive under high concentrations of salinity stress, whereas the viability of wild type cells was completely inhibited at the same high concentrations of salinity stress. In conclusion, the present research documented the expressing of the *sll1621* gene into *E. coli* cells. This result approved the absence of species barrier in relations to the function of *Synechocystis* PCC 6803 PrxII protein in different microorganism.

Keywords: *Synechocystis* PCC6803, *sll1621,*PrxII, salinity stress, *E. coli*

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INTRODUCTION

Peroxiredoxins (Prxs) are a universal group of antioxidant proteins that promote the scavenging of several hydroperoxides[1]. In the past decades, Prxs have gotten extensive consideration as a group of thiol-specific antioxidant proteins. Additionally, they named as thioredoxin peroxidases and/or alkylhydroperoxide reductase proteins[2]. It started back to 1989 when an alkyl hydroperoxide reductase activity was isolated from *Salmonellatyphimurium* and *E. coli.* The enzyme was able to decrease cumene hydroperoxide as an electron acceptor with NADH or NADPH as an electron donor[3]. Yet, in a way autonomous of selenium, glutathione and heme can replace NADH and NADPH4. Thus, in such situation, two enzymes named AhpF (52 kDa) and AhpC (22 kDa) correspondingly made the activity[4]. According to their genetic background and catalytic activities, there are four different groups of enzymes that belong to Prxs which are 1-Cys Prx, 2-Cys Prx, PrxII, and PrxQ[5].

Synechocystis PCC 6803 contains five Prxs genes[6]. These genes are a part of each reported groups as *sll1621* (PrxII), *sll0755* (2-Cys Prx), *slr1198* (1-Cys Prx), and *slr0242*, *sll0221* (Prx Q)[6]. Investigations of cyanobacterial Prxs mutant strains propose that the physiological roles of these proteins are focused on the adjustment of the growth of the cyanobacterial cells at high light intensities, however the catalytic activity may not generally include peroxide detoxification[1,7]. Interestingly, *Synechocystis* PCC 6803 PrxII disrupting mutant strain showed a seriously diminished development and growth rate comparative to the growth rate of wild type cells under even normal light intensities[1,7]. Interestingly, a mutant strain of *Anabaena* PCC 7120 was failed to express one of its four PrxQ, displayed a slow growth rate at moderate light intensities and was hypersensitive to methyl viologen[8]. Perez-Perez *et al.*[9] stated that all five enzymes of *Synechocystis* PCC 6803 Prxs could utilize thioredoxins as an electron donor. The most noteworthy catalytic ability was acquired for the two enzymes of PrxII and TrxQ with thioredoxin as an electron donor[9]. Kobayashi *et al.*[7] indicated that the expression level of *Synechocystis* PCC6803 *sll1621* gene was significantly induced under oxidative stress through the treatment of *Synechocystis* PCC6803 wild-type cells to methyl viologen for 15 min under high light intensities. Additionally, numerous defensive mechanism genes are liable for adaptation under a biotic stress conditions in *Synechocystis* PCC6803 cells. For example, 1-Cys Prx (*slr1198*) and PrxII (*sll1621*) genes are overexpressed under highlight treatment[10]. Also,another antioxidant enzyme in *Synechocystis* PCC6803, NADPH-dependent glutathione peroxidase-like protein (*slr1992*), was found to be overexpressed and enhance the transgenic *Arabidopsis* plants under several abiotic stress including salinity, drought, chilling and highlight[11,12]. Additionally, it was reported that the mRNA levels of *sll1621* under conditions of paraqout-induced oxidative stress increased seven times more than the untreated cells[13]. All these data clearly show the importance of the *sll1621* gene to scavenge the free radicals of reactive oxygen species (ROS) particularly under different abiotic stress conditions such as the exposure to high light and methyl viologen.

The aim of the present researchis to further analyze the effect of the overexpression of the *Synechocystis* PCC 6803 PrxII protein on the growth of *E. coli* BL21 cells under salt stress condition that induced oxidative stress. The present data will help to more emphasizing of the physiological role of *Synechocystis* PCC 6803 PrxII under salt stress and its use for the scavenging of ROS that generated under salt stress.

MATERIALS AND METHODS

Chemicals and bacterial strains

Ampicillin, X-gal and IPTG purchased from UFC Biotechnology (USA). The restriction enzyme *EcoR*I and *E. coli* BL21 strain were acquired from NEB (USA). Other different synthetic compounds were obtained with the highest caliber financially accessible. Cloning vector (pGEM-T easy) was bought from Promega (USA); whereas, the expression vector pTVB21 was gotten from NEB (USA).

Growth conditions

Synechocystis sp. PCC 6803 cells was grown in Allen's medium 14 under dim light condition with shaking for five to seven days. Cloning *E. coli* host cell (DH5α) and the expression *E. coli* host cells (BL21 DE3) were cultured in LB broth media at 36°C. Cells harboring recombinant plasmid was cultured and kept on LB media

accompanied with ampicillin (100 mg/mL).

Gene expression of *Synechocystis* PCC6803 sll1621 in *E. coli*

Total DNA of *Synechocystis* PCC6803 cells was isolated as described previously[15]. The specific *sll1621* gene was isolated using PCR with the following primer: 5'-CATATGACCCCC-GAACGAGTTCC-3' (forward primer) and 5'-CTCGAGTTAGCCG-ACAAAAGCTTTAACG-3' (reverse primer). Both primers were designed to introduce restriction enzymes sites of *Nde*I in forward primer and *Xho*I in reverse primer. The purified PCR product was successfully cloned into *E. coli* DH5a host cells (Promega, USA) using pGEM-T easy vector according to the instruction of the supplier. The positive transformed colony was confirmed by PCR and DNA sequencer. Next, the confirmed sequenced gene was over expressed in *E. coli* BL21 (DE3) (NEB, USA) host cell using *pTYB21* vector (NEB, USA). The resulting construct was designated as *pTYB21*/*sll1621*.

Production of the recombinant protein

The recombinant *pTYB21*/*sll1621*vector was cultured in five mL LB broth accompanied with ampicillin (100 mg/mL)at two different temperatures (16°C or 36°C), then left overnight. Next, the overnight cultures were inoculated to a new 100 mL of LB broth. When the OD_{600} = 0.4, IPTG (400 mmol/L) was added and the culture cells were grown for additional three hours to induce the recombinant protein. In parallel, wild type strain harboring empty *pTYB21* vector was cultured. Both cells were harvested, and SDS-PAGE was achieved in 12% (w/v) as descripted before[14].

Evaluate of salt stress tolerance of recombinant *E. coli* cells

The recombinant (*pTYB21*/*sll1621*) and wild type (*pTYB21*/empty) cells were cultured in 50 mL LB broth accompanied with ampicillin (100 mg/mL) at 36°C with shaking. After the induction of the recombinant protein, both cells were collected by centrifugation at 5000 rpm/10 min. Next, the collected cells were re-inoculated in five ml of LB broth and distributed by equal concentration on LB agar medium including several concentrations of NaCl (0, 400, 800, and 1000mM) and were grown overnight at 36°C.

Protein sequence evaluation

The amino acids sequence of *Synechocystis* PCC 6803 PrxIIwas alignment with other PrxIIproteins from different organisms using ClustalW program[16]. The molecular information of PrxII protein (amino acids composition, molecular weight and pI value)wereestimatedusing the ProtParam resourcesfrom ExPASy website as described previously[15].

RESULTS AND DISCUSSION

Characterization of the *sll1621* gene

To defend the cells and organ against oxidative stress and ROS, aerobic organisms have developed a profoundly classy and complex antioxidant prevention system[17]. It includes a diversity of components, both endogenous and exogenous in source. Both components work collaboratively to remove free radicals[17]. Type II Prxs are one of the most important peroxiredoxins proteins that can catalyze the reduction of various hydroperoxides[1,2]. Investigations of cyanobacterial Prxs mutant cells propose that these proteins work in adjustment to growth and development of cyanobacteria at high-light intensity, however the catalytic activity may not generally contain peroxide detoxification[1]. It was found that the interruption of the *sll1621* gene had a reduction consequence on the survival growth rate of *Synechocystis* PCC 6803 cells under normal or dim light circumstances, suggesting that PrxII enzyme is important for the viability of this cells7. The purpose of the present research is to further explore the effect of the overexpression of *Synechocystis* PCC6803 *sll1621* genein *E. coli*

Table 1. Identity and similarity percent of type II Peroxiredoxin proteins of *Synechocystis* PCC 6803 with other type II peroxiredoxin proteins

Type II	Prx protein from Synechocystis PCC 6803					
	S. PCC 6714	S. PCC 7003	*C. reinhardtii*	*Jatropha curcas*	*Oryza sativa*	*Homo sapiens*
Identity	99%	85%	48%	48%	43.4%	44.2%
Similarity	99%	91%	66%	66%	60.7%	62.8%

cells especially the survive rate under salt stress condition.

The *sll1621* gene was shown to contain of 570 base pair that encoded a protein of 189 amino acids. The molecular mass of the PrxII protein was assessed to be 21.167 kDa with a pI of 4.94. The identity percentage of PrxII protein with other PrxIIs was ranged between 44.2% to 99% (Table 1). Total amino acid sequences of PrxII from different organisms including prokaryotic algae [*Synechocystis* PCC 6714 (189 amino acid) and *Synechococcus* PCC 7003 (187 amino acid)], eukaryotic algae [*Chlamydomonas reinhardtii* (194 amino acid)], higher plants [*Jatropha curcas* (227 amino acid) and *Oryza sativa sub sp. japonica* (225 amino acid)], and human (214 amino acid) were compared with the sequences of *Synechocystis* PCC 6803 PrxII protein (Fig. 1). All PrxIIs proteins share high degree of the critical catalytic cysteine residue inside the active site consensus sequence of PGAFTP(T/G)CS[18]. Moreover, all PrxIIs proteins have two cysteine residues. The second cysteine

Fig. 1. Comparison of the deduced amino acid sequences of *Synechocystis* PCC 6803 type II Prx (189 aa) with those of the type II Prx proteins from *Synechocystis* PCC 6714 (189 aa), *Synechococcus* PCC 7003 (187 aa), *Chlamydomonas reinhardtii* (194 aa), *Jatropha curcas* (227), *Oryza sativa subsp. japonica* (225 aa), and *Homo sapiens* (214 aa). Amino acid sequences were aligned by CLUSTALW method using MAFFT v7.429. The active site is pointed out by black frame, and the two conserved cysteine residues are marked by red asterisk.

residue separated from the first one by the same number of amino acid residues (Fig. 1)[18].

According to the phylogenetic tree analysis of PrxII proteins (Fig. 2), the *Synechocystis* PCC 6803 PrxII was clustered in same group with the similar PrxII proteins from other prokaryotic algae, including *Synechocystis* PCC 6714 and *Synechococcus* PCC 7003. The other PrxII proteins from eukaryotic algae, plants and human were clustered together in second group. Among all of these proteins, *Synechocystis* PCC 6803 PrxII was most nearly to PrxII from *Synechocystis* PCC 6714 (Fig. 2). The PrxII proteins of plants, *Oryza sativa* and *Jatropha curcas*, have an N-terminal extension about 38 amino acids than *Synechocystis* PCC 6803 PrxII (Fig. 1). Also, the human PrxII protein have an N-terminal about 25 amino acids than *Synechocystis* PCC 6803 PrxII. These extensions in plants or human PrxII proteins might be coded for a transit peptide anticipated to address the protein to chloroplast or mitochondria, respectively.

Protein expression of PrxII in *E. coli* cells

We amplify the *sll1621* gene using PCR and the resulted specific product was used for cloning in *E. coli* DH5α cells (Fig. 3). Thesequence of the PCR product (570 base pair, Fig. 3A lane 1) was checkedusing an automated DNA sequencer apparatus. The results of DNA alignments of the gene productwith other genes in NCBI website indicated that the gene was 100% homologous to *Synechocystis* PCC 6803 *sll1621* gene (data not shown). To further ensure about the full sequence of *sll1621* gene, we performed a restriction gene map using *Eco*RI restriction enzyme. As expected, the restriction map of the *sll1621* after cutting with *Eco*RI was given three fragments with lengths of 259 bp, 229 bp and 123 bp(Fig. 3A, lane 2).

The restriction sites of NdeI and XhoI that involved in the gene product were used to overexpress *sll1621* gene into *pTYB21* vector. The resulting was namedas*pTYB21*/*sll1621*, which was transformed into *E. coli* BL21 (DE3) cells to obtain there combinant protein. Subsequently, we examined the best conditions for the growth of BL21 (DE3) cells to produce PrxII protein with high expression (Fig. 3B). In this case two types of temperatures were used (15°C and 36°C). The reason for choosing 15°C, as it is recommended for the overexpression of the Intein protein, of the *Saccharomyces cerevisiae*, that fused at the N-terminus of *pTYB21* vector (56 kDa). The recombinant protein was successfully expressed with a sufficient level in BL21 (DE3) cells at 36°C (Fig. 3B). The predicted molecular weight of

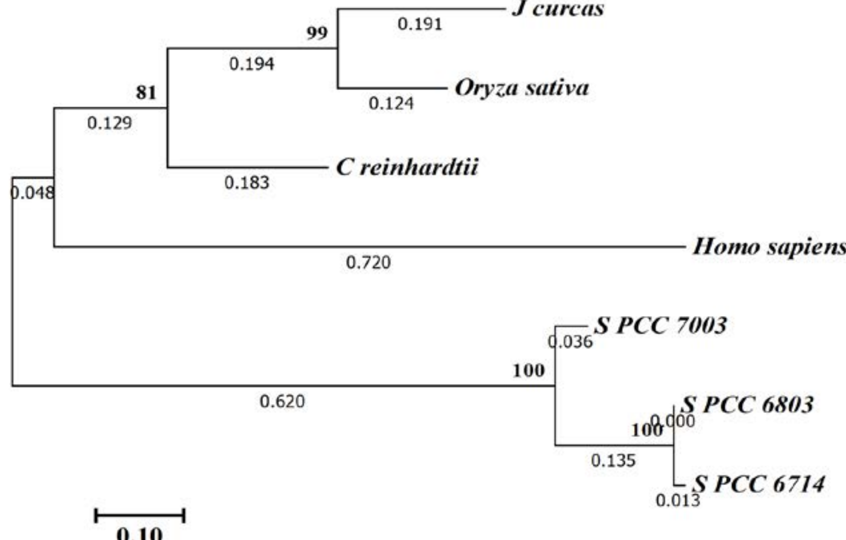

Fig. 2. Molecular phylogenetic tree by Maximum Likelihood method of the *Synechocystis* PCC 6803 type II Prx protein (S. PCC 6803) with those of the type II Prx proteins from other organisms including *Synechocystis* PCC 6714 (S. PCC 6714), *Synechococcus* PCC 7003 (S. PCC 7003), *Chlamydomonas reinhardtii, Jatropha curcas, Oryza sativa,* and *Homo sapiens*. The bootstrap consensus tree was from 1000 replicates. Numbers by nodes indicate Maximum Likelihood bootstrap. Evolutionary analyses were conducted in MEGA7[28].

the recombinant PrxII protein (21.167 kDa) was detected (Fig. 3B, lane 3). Whereas, we could not detect any protein band related to PrxII molecular weight in *E. coli* control cells (Fig.3B, lane 4). Interestingly, at 15°C, we detect a protein with a molecular weight of 77 kDa in recombinant BL21 (DE3) cells that harboring *pTYB21*/*sll1621* vector (Fig. 3B, lane 2). This molecular weight is considered for the total molecular weight of the Intein protein (56 KDa) that expressed by *pTYB21* at 15°C plus PrxII protein (21.16 KDa).

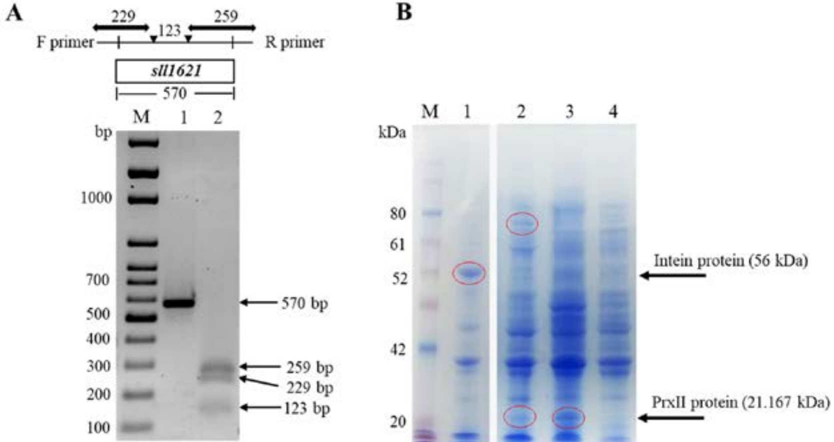

Fig. 3. Isolation and expression of *sll1621* gene in *E. coli* BL21 (DE3) cells. (A) PCR amplification of *sll1621* gene that isolated from the genomic *Synechocystis* PCC 6803 (lane 1),while, lane 2 represented the restriction map of the *sll1621* gene using *Eco*RI. (B) SDS-PAGE analysis of the recombinant PrxII protein after addition of IPTG. Lane 1, *E. coli* cells transformed with *pTYB21*/empty vector that cultured at 15°C for 16 hours; lane 2,*E. coli* cells transformed with pTYB21/sll1621vector that cultured at 15°C for 16 hours; lane 3, *E. coli* cells transformed with *pTYB21/sll1621* vector that cultured at 36°C for 3 hours; lane 4, E. coli cells transformed with *pTYB21*/empty vector that cultured at 36°C for 3 hours; lane M, PiNK plus prestained protein ladder (GeneDireX).

***Synechocystis* PCC 6803 PrxII protein is necessary for improve growth of *E. coli* under salt stress**

Peroxiredoxins are proteins that have molecular weight around 20 to 30 kDa and widely spread in living organisms[19]. Initially, Prxs were identified according to its ability to protect cells especially proteins from abiotic stress particularly oxidative damage that leads to an increase of ROS[20]. Previously, Prxs was termed as the "protector protein" or "thiol-specific antioxidant" before being name again as Prxs[21-24]. Five different Prxs genes including *sll1621* were found in the genome of *Synechocystis* PCC 68036. Also, Kobayashi *et al.*[7] stated that the mRNA level of the *sll1621* gene is remarkably up-regulated when *Synechocystis* PCC 6803 are exposed to methylviologen with light conditions. Also, Hosoya-Matsuda *et al.*[1] concluded that PrxII is significantly important as scavenging ROS enzyme. They found that the growth of the knockout mutant cells lacking *sll1621* gene was remarkably weak against oxidative stress[1].

Oxidative injury to microorganism cells frequently happens under different abiotic stress conditions during bioprocess[11,15]. Thus, it has been addressed either the peroxiredoxin activity of PrxII protein assumes a significant useful role in recombinant *E. coli*, and additionally whether such activity might be useful under salinity stress. In the present research, we used the model microorganism, *E. coli*, to examine the ability of *Synechocystis* PCC 6803 *sll1621* gene to increase the viability of growth of *E. coli* recombinant cells under salt stress. Salt stress was generated in-vitro by culturing the recombinant and wild type *E. coli* cells on LB agar provided with various concentrations of NaCl (Fig. 4). The *pTYB21*/empty cells were sensitive to 400mM NaCl, while, the growth was completely deleted in the presence of 800mM and 1000mM of NaCl (Fig. 4). On the other hand, the recombinant *pTYB21*/*sll1621* cells increased the growth efficiency under the high concentration of NaCl (1000mM), demonstrating that the growth was, for some reason, improved

compare to those of the *pTYB21*/emptycells (Fig. 4). Accordingly, this survival test under different concentration of NaCl showed that the *sll1621* gene from *Synechocystis* PCC 6803 could function as an antioxidant protector enzyme to defend cells from oxidative damage under salt stress (Fig. 4). Similar results documented that the expression of PrxIIgene are typically affected by oxidative stress and abiotic stress that caused by hyperoxia, peroxides, UV, and ionizing radiation[25-27].

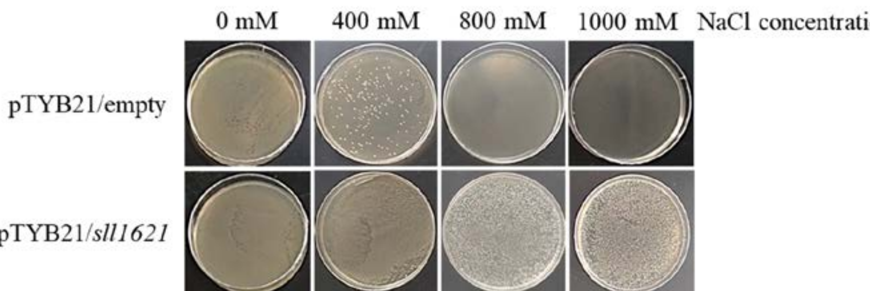

Fig. 4. Impact of various concentrations of NaCl on the growth of recombinant *pTYB21/ sll1621* and *pTYB21*/empty *E. coli* cells. Full conditions for experiments are depicted in ''Materials and Methods section''.

CONCLUSIONS

In the present work, the *Synechocystis* PCC 6803 *sll1621* gene was successfully over expressed in the cytoplasm of *E. coli* BL21 (DE3) cells. The over expressed protein was verified by SDS-PAGE and observ ableviability test. The present data specify that *Synechocystis* PCC 6803 *sll1621* gene confers tolerance of recombinant *E. coli* cells to high concentration of salt stress. Consequently, these results are strong evidence for the success of gene expression and the absence of species barrier among different microorganisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

AG, MMF, AKI and MHH involved in the design of the research. AG and MHH designed the experimental procedure. AG performed the molecular cloning and gene expression experiments. MMF and AKI involved in SDS-PAGE experiment. AG wrote the original draft. MMF, AKI, MHH, SA and WFA involved in analysis the data. MMF, AKI, MHH, SA and WFA reviewed and edited the final version. All authors agreed with the final version of the manuscript.

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ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY

All datasets analyzed in the study are involved in the manuscript and existing as tables and figures.

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