Comparative analysis of cyanobacterial superoxide dismutases to discriminate canonical forms
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Abstract

Background: Superoxide dismutases (SOD) are ubiquitous metalloenzymes that catalyze the disproportion of superoxide to peroxyde and molecular oxygen through alternate oxidation and reduction of their metal ions. In general, SODs are classified into four forms by their catalytic metals namely; FeSOD, MnSOD, Cu/ZnSOD and NiSOD. In addition, a cambialistic form that uses Fe/Mn in its active site also exists. Cyanobacteria, the oxygen evolving photosynthetic prokaryotes, produce reactive oxygen species that can damage cellular components leading to cell death. Thus, the co-evolution of an antioxidant system was necessary for the survival of photosynthetic organisms with SOD as the initial enzyme evolved to alleviate the toxic effect. Cyanobacteria represent the first oxygenic photoauto trophs and their SOD sequences available in the databases lack clear annotation. Hence, the present study focuses on structure and sequence pattern of subsets of cyanobacterial superoxide dismutases.

Result: The sequence conservation and structural analysis of Fe (Thermosynechococcus elongatus BP1) and MnSOD (Anabaena sp. PCC7120) reveal the sharing of N and C terminal domains. At the C terminal domain, the metal binding motif in cyanoprokaryotes is DVWEHAYY while it is D-X-[WF]-E-H-[STA]-[FY]-[FY] in other pro- and eu karyotes. The cyanobacterial FeSOD differs from MnSOD at least in three ways viz, (i) FeSOD has a metal specific signature F184X3A188Q189.......T280......F/Y303 while, in Mn it is R184X3G188G189......G280......W303, (ii) aspartate ligand forms a hydrogen bond from the active site with the outer sphere residue of W243 in Fe where as it is Q262 in MnSOD; and (iii) two unique lysine residues at positions 201 and 255 with a photosynthetic role, found only in FeSOD. Further, most of the cyanobacterial Mn metalloforms have a specific transmembrane hydrophobic pocket that distinguishes FeSOD from Mn isoform. Cyanobacterial Cu/ZnSOD has a copper domain and two different signatures G-F-H-[ILV]-H-x-[NGT]-[GPDA]-[SQK]-C and G-[GA]-G-G-[AEG]-R-[FIL]-[AG]-C-G, while Ni isoform has an nickel containing SOD domain containing a Ni-hook HCDGPCVYDPA.

Conclusion: The present analysis unravels the ambiguity among cyanobacterial SOD isoforms. NiSOD is the only SOD found in lower forms; whereas, Fe and Mn occupy the higher orders of cyanobacteria. In conclusion, cyanobacteria harbor either Ni alone or a combination of Fe and Ni or Fe and Mn as their catalytic active metal while Cu/Zn is rare.
Background
Superoxide dismutases (SODs, E.C. 1.15.1.1) are the superfamily of metalloenzymes that dismutate the highly toxic and reactive superoxide radical (O$_{2}^{\cdot-}$, by-product of aerobic metabolism) through a cyclic oxidation-reduction (‘ping-pong’) mechanism. As described by McCord and Fridovich [1], it is the first line of defense to alleviate oxidative stress virtually in all living organisms that survive in oxic environment.

The evolutionary trajectory has favored SOD as a ubiquitous enzyme in multiple forms within a single organism or cell, indicating a fail-safe redundancy that emphasizes the importance of this family of enzymes against reactive oxygen species (ROS). Based on metal cofactors, four known (canonical) isoforms viz., iron (Fe), manganese (Mn), copper/zinc (Cu/Zn) and nickel (Ni) SODs have been identified. In general, SODs have a strict metal binding specificity for enzymatic activities with the exception of a class of enzymes which show enzymatic activity regardless of whether Fe or Mn is bound at the active site; these are known as cambialistic forms [2-5].

Cyanoprokaryotes are oxygen evolving photosynthetic organisms occupying a crucial position between pro- and eukaryotes. They are considered to be primeval having evolved about 3.2 billion years ago [6]. In addition, they succeeded in linking photosynthetic electron flow from water as the photoreductant through an oxygen-evolving complex at the high-potential side of the newly elaborated photosystem II, which is thought to have originated from a uniform primordial photosystem by gene duplication [7]. The resultant tandem operation of two photosystems is now known as oxygenic or plant-type photosynthesis [8]. This marked the turning point in the evolution of earth, opening up the era of an aerobic, oxygen-containing biosphere and SOD is found to play a critical role in mitigating the toxic effect of superoxide ion. The first implication on the protective role of cyanobacterial SOD in photo-oxidative damage was shown in Anacystis nidulans [9]. Subsequently, several studies on protective role of SODs of cyanobacteria in response to various physiological processes/stresses like photosynthesis [10], desiccation [11,12], chilling [13], nitrogen starvation [14] and with azo dyes (unpublished) have been reported.

Metal preferences in Fe and MnSODs have been well documented in both pro- and eukaryotic forms [15-17]. However, no information is available on distinguishing the canonical isoforms of cyanobacteria. Hence, the present study focuses on structure and sequence pattern of subsets of cyanobacterial SODs to explore the possibility of solving the ambiguity.

Results and Discussion
For the survival of cyanobacteria with oxygenic photosynthesis, the selection pressure led to the evolution of SODs as the first antioxidant arsenal against nascent oxygen species. Studies on cyanobacterial SODs would serve as a window into the past and present evolutionary events of these primitive phototrophs.

On comparison, the canonical isoforms of SOD, Fe and MnSOD’s are structurally distinct from Cu/Zn and NiSOD. Both Fe and MnSOD are typically homodimers or tetramers (Fig 1A,C) sharing identical metal chelating residues at the active site with a high degree of sequence and structural homology except for slight differences in amino acid residues. For instance, the amino acid range in cyanobacterial FeSOD is 199–229 residues with a molecular weight of 21–25 KDa, whereas in MnSOD, it is 200–316 amino acids with a molecular weight of 22–34 KDa.

Both SODs revealed a common topology with all α N-terminal (Pfam:PF00081) and a α/β C terminal domains (Pfam:PF02777) (Fig 1B,D). The sequence pattern for Fe and MnSODs of eukaryotes and other non-cyanobacterial prokaryotes is D-X-[WF]-E-H-[STA]-[FY]-[FY] [18];
whereas, the analysis of the sequence conservation in cyanobacteria (based on available data) showed a specific motif DVWEHAYY [D282-Y289, based on Fig 2]. This motif extends between the second α-helix and the first β-sheet of the C-terminal domain in both the SOD’s. The highly conserved residues aspartate D282 and histidine H286, a constituent of the motif are the metal binding ligands. In addition, glutamic acid E285 and tyrosine Y289 form a dimer surface spanning the interface and bridging the active sites between the opposite halves of each subunit, see Figure 2 (For full image, please see Additional file 1).

Structural analysis of available cyanobacterial Fe and MnSODs, confirms that both share a similar active site (i.e., metal ion) being coordinated in the respective isoform by three histidine and an aspartate residue with a ligating solvent molecule (water or OH), a five coordinated trigonal bipyramidal geometry. In *Thermosynechococcus elongatus* (PDB code 1my6); the Fe ion is coordinated by the carboxylate oxygen (Oδ2) of D161 with the amino group (Nε2) of H79, 27, 165 along with the oxygen atom of the water molecule. The hydrogen bonding distance between Oδ2 (D161) and Nε2 (H27 and H79) is 2.79Å and 3.27Å respectively (Table 1). In case of *Anabaena* sp (PDB code: 1gv3), the Mn is coordinated by Nε2 of H117, 204, 62 and Oδ2 of D200. The hydrogen bonding between Oδ2 (D200) and Nε2 (H62 and H117) is 2.19Å and 3.33Å respectively. These hydrogen bonds are involved in stabilizing the orientation of the ligand residues in MnSOD [8]. The observed contact surface area (31–35 Å²) between the side chain aspartate oxygen atom (Oδ2) and histidine (Nε2) implies that the metal coordination ligands in the exposed region may perhaps tune the redox potential (Fig 3, 4).

The motif and metal binding sites of Fe and Mn isoforms appear to exhibit similar function. However, the sequence alignment and structural analysis reveal their possible discrimination by three traits to specifically differentiate Fe and Mn isoforms (Table 1 Additional file 1).

First, is the change in conserved amino acid signature F184X_A188Q189__T280__F/Y303 in Fe being replaced by R184X_G188G189__G280__W303 in MnSOD (see Figures 2 and 5).

The second notable feature is related to the metal bound solvent molecule that serves as a hydrogen bond to the non-coordinated oxygen of the carbonyl group of the aspartate ligand accepting a hydrogen bond from an outer sphere residue [19]. In MnSOD, it is glutamine Q262 (Fig 2) arising from the end of the βγ-strand and H9 in the C-terminal domain, while in FeSOD, it is tryptophan W243 arising from the middle of the sequence (within the βγ) in the C-terminal domain. In the case of cambialistic Fe/ MnSOD metalloform reported in archaea (*Pyrobaculum aerophilum*) [19], the outer-sphere H-bonding residue is histidine. This residue plays a major role in altering the solvent interaction with the active site metal ion in cambialistic Fe/Mn SOD isoform [19]. The sequence analysis of cyanobacterial SODs showed the absence of this histidine residue which probably suggests the absence of cambialistic forms in cyanobacteria. Vance and Miller [20] reported that the most highly conserved residues glutamine Q262 in Mn and Q189 of FeSOD forms the outer sphere hydro-

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**Figure 2**

This figure shows the lower quartile of protein sequence alignment of Fe and MnSODs in cyanobacteria. The highly conserved metal specific residues are highlighted in red for Fe and green for MnSODs. Residues involved in outer sphere hydrogen bonding for Mn is highlighted in cyan and for Fe in orange. For FeSOD, the lysine residues involved in photosynthetic context is shown in pink. The active site residues are marked as I and the dimer residues are represented by *.
gen-bond network exerts a large influence on redox mid-point potential tuning for catalytic activity of SOD's.

The third difference is the presence of two lysine residues, K201 and 255 in FeSOD but not in MnSOD (Fig 2 and 5). These residues seem to be unique and function specific to cyanobacteria among prokaryotes [21]. K201 lines a small pit at the surface of the \emph{T. elongatus} and of higher plants FeSOD, formed by the loop P202-G203-G204 connecting N and C terminal domains. Likewise, K255 is restricted only to cyanobacteria, indicating its importance in the photosynthetic context [21].

Cyanobacterial MnSOD is the only SOD to be membrane anchored by transmembrane helix [22]. The factor that determines localization of MnSOD is found to span the N terminal which is a hydrophobic transmembrane helix (Fig 1D, 6). The cyanobacterial representatives such as (\emph{Synechococcus} sp. WH5701 (EAQ76095), \emph{Synechococcus} sp. RS9917 (EAQ68777), \emph{Trichodesmium erythraeum} IMS101 (EAO27349), \emph{Anabaena variabilis} ATCC29413 (ABA21068) and \emph{Nostoc} sp. PCC7120 (BAB77594)) clearly corroborate this (Fig 6).

Cyanobacterial Cu/ZnSOD isoform bears no resemblance to Fe or Mn or Ni isoform in relation to its primary and tertiary structure. The theoretical molecular weight ranges between 16–23 KDa with an amino acid length of 174–233 residues. Further, study on amino acid composition illustrates that it is rich in Gly (11–16%) forming eight \(\beta\)-sheets (Fig 7A) accredited to be involved in confor-

Table 1: Discriminatory key to classify indecisive isoforms.

| Characteristics                        | FeSOD | MnSOD |
|----------------------------------------|-------|-------|
| Metal specificity                      | Fe    | Mn    |
| Amino acid length                      | 199–229 | 200–316 |
| Theoretical molecular weight           | 21–25 KDa | 22–34 KDa |
| No. of a helix*                        | 13    | 14    |
| No. of b strand*                       | 3     | 3     |
| Domains                                | N & C terminal | N & C terminal |
| Motif                                  | DVWEHAYY | DVWEHAYY |
| Active site residues*                  | Fig 3 | Fig 4 |
| Structurally highly conserved metal specific residues | K87, K139 | R184XXXG189G189...G280...W303 |
| Conserved residue with photosynthetic role | Absent | None |
| Transmembrane hydrophobic pocket       | Absent | Present |

* – Based on the structural analysis of MnSOD of \emph{Anabaena} sp. (PDB No: 1gv3) and FeSOD of \emph{Thermosynechococcus elongatus} BP-1 (PDB No: 1my6)
mation [23] and stability in repeated freeze/thaw cycles and prolonged refrigeration [9]. These isoforms in general have a copper containing domain (Pfam:PF00080) with two different signatures. The first is G-F-H-[ILV]-H-x-[NGT]-[GPDA]-[SQK]-C where the conserved histidine is involved in copper binding, and the second being G-[GA]-G-G-[AEG]-R-[FIL]-[AG]-C-G where C is involved in disulfide bonding (Fig 8).

**G. violaceus** SOD (NP_925116, NP_924927) annotated as 'similar to SOD' contains only copper binding domain and both the signatures are absent. Further confirmation requires additional structural data. Each monomer is comprised of a binuclear metal centre with one Cu and one Zn atom. The noticeable β parallel fold of cyanobacterial Cu/Zn isoform mimics the structure of *Salmonella typhimurium* Cu/ZnSOD [24] (Fig 7B). The catalytic coordination sphere of Cu$^{2+}$ ion is by Nδ1 of H103, Nε2 of H105, H147 and H215 and Zn$^{2+}$ by Nδ1 of three H147, 157, 171 and Oδ1 of one D174 (Fig 8). Besides this, structural comparison designates the two specific hydrogen bonds between the Zn$^{2+}$ coordinating residues D174-Oδ1...H157-Nδ1 (3.25 Å) and D174-Oδ1...H171-Nε1 (3.18 Å) to ligand stability.

The fourth canonical form NiSOD is a hexamer (Fig 9A) found only in cyanobacteria [25] and *Streptomyces* [26,27] with amino acids ranging from 140–163 and molecular weight between 15–18 KDa. Analysis of available sequences and complete genome sequences revealed that, unicellular *Prochlorococcus* forms possess only NiSOD, whereas, multicellular filamentous heterocystous and heterotrichous forms lacks this isoform (Table 2). The key for the ubiquity of NiSOD in *Prochlorococcus* may be due to
the primitive photosynthetic machinery and its smallest genome size (between 1669–2434 Kb) by gene rearrangement or loss to maximize the energy economy [28]. The sequence conservation, motif with eleven-residues (HCDGPCVYDPA) in N-terminal region of Ni-hook, along with a nickel containing SOD domain (Pfam:PF09055) forms an unique pattern to identify cyanobacterial NiSOD. Cyanobacterial NiSODs seem to have an assembly of four alpha helices bundle with a short connecting alpha helix, as that of Streptomyces sp. (Fig 9B). The catalytic Ni ion of cyanobacteria is very much analogous to the reported square planar active center with thiolate (C2, based on 1t6u), backbone nitrogen (H1 and C6) ligands and of square pyramidal Ni (III) with an added axial His, side chain of Streptomyces sp. [29].

**Conclusion**

The analysis is based on 64 cyanobacterial SODs available to date in public databases. Among them 2 are described

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**Figure 7**
Representative structure of *Salmonella typhimurium* Cu/Zn superoxide dismutase. (a) Tetrameric subunits of Cu/ZnSOD. Chain A coded in green, B in pink, C in yellow and D in cyan. (b) Crystallographic structure of functional S. typhimurium Cu/ZnSOD (PDB 1eqw) subunit is represented to highlight the active site residues in ball and stick mode visualized using WebLab ViewerLite 4.2 software.

**Figure 8**
Sequence alignment of cyanobacterial copper zinc superoxide dismutase with bacterial representatives. Alignment was carried out using Clustal W of BioEdit Package (v.7.0.5) [28]. The active site Cu residues are marked as * and Zn in #. The signature 1 residues are highlighted in green and signature 2 in blue.
### Table 2: Annotation of cyanobacterial superoxide dismutases based on sequence and structure conservation.

| Organisms                        | Accession no | Sequence length | Type of SOD in Database | Confirmed isoform from our study |
|----------------------------------|--------------|----------------|-------------------------|----------------------------------|
| Prochlorococcus marinus AS9601   | YP_001009883| 157            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus CCMP1996 | NP_89311     | 156            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus CCMP1375 | NP_875759    | 157            | Ni                      | NiSOD                            |
| Prochlorococcus marinus MIT 9301 | YP_00109170 | 157            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus MIT 9303 | YP_00107980 | 164            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus MIT 9211 | ZP_01004940 | 140            | Ni                      | NiSOD                            |
| Prochlorococcus marinus MIT 9312 | YP_397886    | 157            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus MIT 9313 | NP_89411     | 157            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus MIT 9515 | YP_0011769   | 157            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus NATL1A   | YP_001017980| 164            | putative Ni             | NiSOD                            |
| Prochlorococcus marinus NATL2A   | YP_0010155334| 163            | putative Ni             | NiSOD                            |
| Synechococcus sp. WH 8102        | NP_897719    | 157            | Ni                      | NiSOD                            |
| Synechococcus sp. BL107          | ZP_01469600  | 198            | putative SOD            | Cu/ZnSOD                         |
| Synechococcus sp. CC9605         | ZP_01468043  | 157            | putative Ni             | NiSOD                            |
| Synechococcus sp. CC9311         | YP_729969    | 175            | Cu/Zn                  | Cu/ZnSOD                         |
| Synechococcus sp. CC9301         | YP_730975    | 155            | Ni                      | NiSOD                            |
| Synechococcus sp. CC9902         | YP_376992    | 175            | putative SOD            | Cu/ZnSOD                         |
| Synechococcus sp. WH 8501        | ZP_00517273  | 159            | Hypothetical protein    | NiSOD                            |
| Synechococcus sp. WH 8501        | ZP_00514026  | 254            | SOD                    | MnSOD                            |
| Synechococcus sp. WH 7805        | YP_171447    | 229            | SOD                    | FeSOD                            |
| Synechococcus sp. WH 7805        | ZP_01472508  | 177            | SOD precursor (Cu-Zn)  | Cu/ZnSOD                         |
| Synechococcus sp. JA-3-3Ab       | YP_476321    | 199            | Fe                     | FeSOD                            |
| Synechococcus sp. JA-2-3B'a(2–13)| YP_478710    | 199            | Fe                     | FeSOD                            |
| Synechococcus sp. WH 7805        | ZP_01124652  | 199            | SOD                    | FeSOD                            |
| Synechococcus sp. WH 7805        | ZP_01123794  | 174            | putative SOD            | Cu/ZnSOD                         |
| Synechococcus sp. WH 7805        | ZP_01084003  | 199            | SOD                    | FeSOD                            |
| Synechococcus sp. RS9916         | ZP_01084015  | 231            | Mn                     | MnSOD                            |
| Synechococcus sp. RS9916         | ZP_01470625  | 199            | SOD                    | FeSOD                            |
| Gloeobacter violaceus PCC 7421   | NP_927273    | 203            | SOD                    | FeSOD                            |
| Gloeobacter violaceus PCC 7421   | NP_923628    | 316            | SOD                    | MnSOD                            |
| Gloeobacter violaceus PCC 7421   | NP_924927    | 233            | similar to SOD         | NA*                              |
| Gloeobacter violaceus PCC 7421   | NP_925116    | 191            | similar to SOD         | NA*                              |
| Synechococcus sp. RS9917         | ZP_01081353  | 199            | SOD                    | FeSOD                            |
| Synechococcus sp. RS9917         | ZP_01080487  | 229            | SOD                    | MnSOD                            |
| Cyanothece sp. CCY0110           | ZP_01728505  | 200            | SOD                    | FeSOD                            |
| Thermostynechococcus elongatus BP-1| NP_682309   | 200            | SOD                    | FeSOD                            |
| Lyngbya sp. PCC8106              | ZP_0169885   | 201            | SOD                    | Cu/ZnSOD                         |
| Lyngbya sp. PCC8106              | ZP_01619231  | 201            | SOD                    | FeSOD                            |
| Trichodesmium erythraeum IMS101  | YP_723986    | 254            | SOD                    | MnSOD                            |
| Trichodesmium erythraeum IMS101  | YP_720765    | 159            | putative Ni             | NiSOD                            |
| Synechocystis sp. PCC 6803       | NP_491347    | 199            | Fe                     | FeSOD                            |
| Spirulina platensis              | AAA22734     | 170            | Fe                     | FeSOD                            |
| Plectonema boryanum UTEX 485     | AAA69954     | 199            | Fe                     | FeSOD                            |
| Plectonema boryanum UTEX 485     | AAA69953     | 239            | superoxide dismutase [Mn] precursor | MnSOD                            |
| Plectonema boryanum UTEX 485     | AAA69950     | 248            | MnSOD                  | MnSOD                            |
| Plectonema boryanum UTEX 485     | AAA69952     | 206            | MnSOD                  | MnSOD                            |
| Leptolyngbya valderiana BDU20041 | AAA84682     | 144            | Mn                     | MnSOD                            |
| Nostoc punctiforme PCC 73102     | ZP_00108516  | 200            | SOD                    | FeSOD                            |
| Nostoc punctiforme PCC 73102     | ZP_00112125  | 249            | SOD                    | MnSOD                            |
| Nostoc punctiforme PCC 73102     | ZP_00108372  | 259            | SOD                    | MnSOD                            |
| Nostoc sp. PCC 7120              | QBY8SZ1      | 200            | Fe                     | FeSOD                            |
| Nostoc sp. PCC 7120              | ADS1417      | 200            | Fe                     | FeSOD                            |
as Fe/Mn, 4 as Cu/Zn and Mn precursor, 16 as putative NiSOD, 11 annotated as Fe, Mn and Cu/Zn isoforms, 29 as possible/putative SOD and 2 as hypothetical proteins.

Thus the present study resolves the incompletely annotated SODs among cyanobacteria (Table 2). Further, 64 cyanobacterial SOD sequences are clearly categorized into 17 NiSOD, 7 Cu/ZnSOD, 24 FeSOD and 14 MnSOD genes, 2 non assignable as they require further structural data. The strict metal specificity, precise sequence and structure among the metalloforms led to discriminate Mn and FeSOD (Table 1). The highly homologous Fe and MnSODs shares a metal binding motif DVWEHAYY without any variation, compared to D-X-[WF]-E-H-[STA]-[FY]-[FY] found in other pro – and eukaryotes.

The whole genome sequences analyses of cyanobacteria reveal that the primitive unicellular Prochlorococcus with simple photosynthetic apparatus possesses only NiSOD. The more evolved middle order forms of cyanobacteria posses a combination of Fe and Ni or Fe and Mn SODs. The most evolved filamentous, heterotrichous and heterocystous forms predominantly have only Fe and Mn metalloforms. However, CuZn also occurs rarely (Table 2).

### Methods

The non-redundant database of protein sequences (National center for Biotechnology Information, NIH, Bethesda) were retrieved using the PHI-BLAST [30] search tool using BLOSUM 62 matrix with gap penalties (Existence – 11 and Extension – 1) with a threshold value of 0.005 and optimal limit for cyanobacteria. The query sequence used were *Synechococcus* sp. JA-3-3Ab with Expasy-PROSITE pattern D-x-[WF]-E-H-[STA]-[FY]2 for Fe/MnSOD; *Synechococcus* sp. RSS9916 with signature 1 [GA]-[IMFAT]-H-[LIVF]-H-[S]-x-[GP]-[SDG]-x-[STAGDE] and signature 2 (G-[GNHD]-[SGA]-[GR]-x-R-x-[SGAWRV]-C-X(2)-[IV]) for Cu/ZnSOD. In addition, the individual sequences of all the SOD metalloforms were also manually retrieved from public databases (NCBI, KEGG). Identical sequences from the same organism were removed manually. *Intoto*, 64 sequences representing 24 complete genomes and individual submissions obtained are listed in Table 2 together with the accession numbers and the organisms. Identification of domains associated with SOD proteins were realized using NCBI Conserved Domain Search and Pfam servers.

The secondary structure consensus was carried out using npPREDICT [31] and JPRED [32] for each protein to refine the multiple sequence alignment. Multiple alignments for cyanobacterial Fe and MnSODs; and Cu/ZnSOD sequences were generated using the Clustal W (neighbor-joining) of BioEdit V.7.0.5 [33] program. Default parameter for both the alignments was gap initial penalty- 8 and gap extension penalty of 2. The alignment was fixed under the PAM40 series protein-weight matrices in both the cases. The sequence alignments were displayed graphically using BIOEDIT package [28] with a threshold of 95% consensus residue shading.

Representative crystal structures of available cyanobacterial FeSOD (1my6-Thermosynechococcus elongates BP-1) and MnSOD (1gv3-Anabaena sp. PCC7120) with exception for NiSOD (1t6u-Streptomyces coelicolor) and Cu/ZnSOD (1eqw-Salmonella typhimurium) were retrieved from PDB. The 3D structures were analyzed using SWISS-PDB viewer [34] and graphical representations were done with WebLab viewer lite (V.4.2)
Authors’ contributions

BP and JP contributed equally in carrying out the sequence analysis studies and participated in the sequence alignment. RTD carried out further confirmation of the results and helped BP in visualization of the structures. TS helped in carrying out the structural comparison. LJU and DP participated equally in the study, its design and coordination. GS helped in fine tuning of the manuscript. All authors read and approved the final manuscript written by BP.

Additional material

Additional file 1

Excerpts of aminoacid sequences of Fe and MnSOD of cyanobacteria. The proteins are labeled by their accession number with organism source and the metal cofactor specificity. Conserved residues for discrimination of Fe and Mn metalloforms in cyanobacteria based on multiple alignment using ClustalW of BioEdit Package [v.7.0.5] [28]. The highly conserved metal specific residues are highlighted in red for Fe and green for MnSODs. Transmembrane hydrophobic pocket specific for membrane binding in MnSOD at the N-terminal region is highlighted in violet. Residues involved in outer sphere hydrogen bonding for Mn is highlighted in cyan and for Fe in orange. For FeSOD, the lysine residues involved in photosynthetic context is shown in pink. The active site residues are marked as I and the dimer residues are represented by *. Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2164-8-435-S1.jpeg]

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