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Comparative analysis of cyanobacterial superoxide dismutases to discriminate canonical forms

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Published: 27 November 2007

Received: 8 May 2007

BMC Genomics 2007, 8:435 doi:10.1186/1471-2164-8-435

Accepted: 27 November 2007

This article is available from: <http://www.biomedcentral.com/1471-2164/8/435>

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Abstract

Background: Superoxide dismutases (SOD) are ubiquitous metalloenzymes that catalyze the disproportion of superoxide to peroxide 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 photoautotrophs 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* BPI) 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 eukaryotes. 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 a 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 dismutates the highly toxic and reactive superoxide radical (O_2^- , 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];

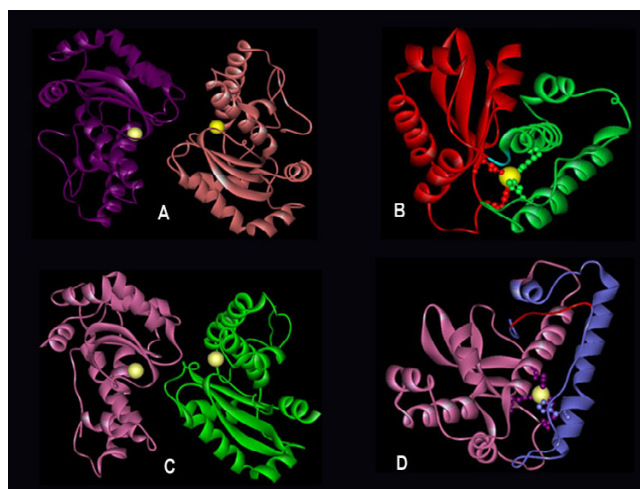


Figure 1

Structure of Fe and MnSOD. Structures are visualized using WebLab ViewerLite 4.2 software. Catalytically essential aspartate or histidine residues are represented in ball and stick mode binding the active metal (yellow) is shown to identify the location of the active site. Protein database codes are given in parentheses: (i) FeSOD (PDB 1gv3); (ii) MnSOD (PDB 1my6). (A) FeSOD of *T. elongatus* BP-I dimers are distinguished by colour (violet and slate), and structures are represented with the active site (yellow) of subunit. (B) Monomeric subunit of FeSOD represents an N-terminal (green) and a C-terminal (red). Similarly (C) represents dimer structure of *Anabaena* sp. MnSOD in pink and green with active site highlighted in yellow. (D) Monomeric MnSOD showing the N-terminal residues in blue and C-terminal in pink with metal binding ligands. The transmembrane hydrophobic pocket specific for MnSOD is highlighted in red (D).

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 (O δ 2) of D161 with the amino group (N ϵ 2) of H79, 27, 165 along with the oxygen atom of the water molecule. The hydrogen bonding distance between O δ 2 (D161) and N ϵ 2 (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 N ϵ 2 of H117, 204, 62 and O δ 2 of D200. The hydrogen bonding between O δ 2 (D200) and N ϵ 2 (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 (O δ 2) and histidine (N ϵ 2) 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 F184X₃A188Q189.....T280.....F/Y303 in Fe being replaced by R184X₃G188G189.....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 β ₂-strand and H₉ in the C-terminal domain, while in FeSOD, it is tryptophan W243 arising from the middle of the sequence (within the β ₁) 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-

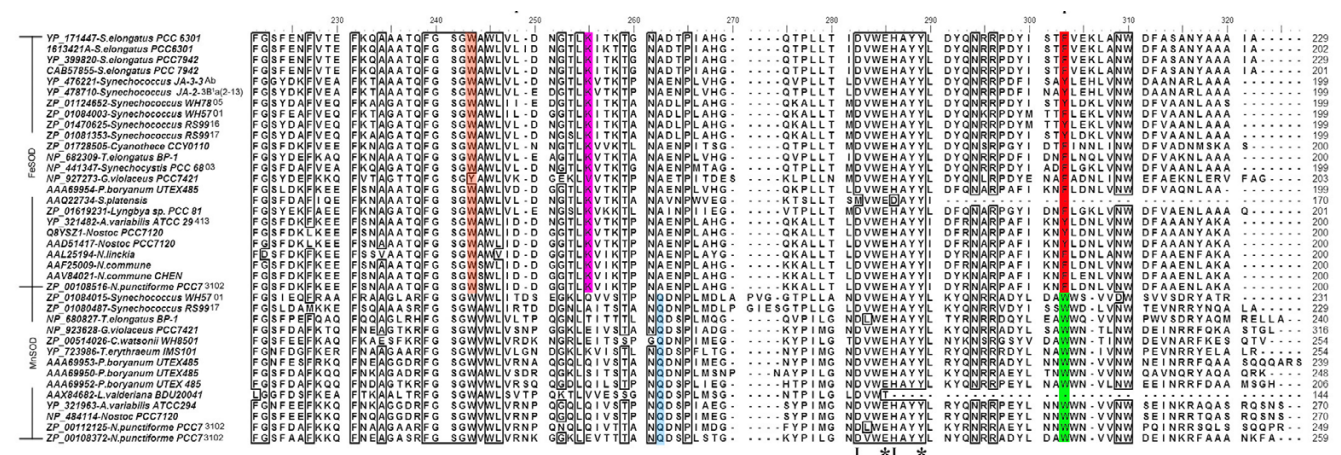


Figure 2

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 *.

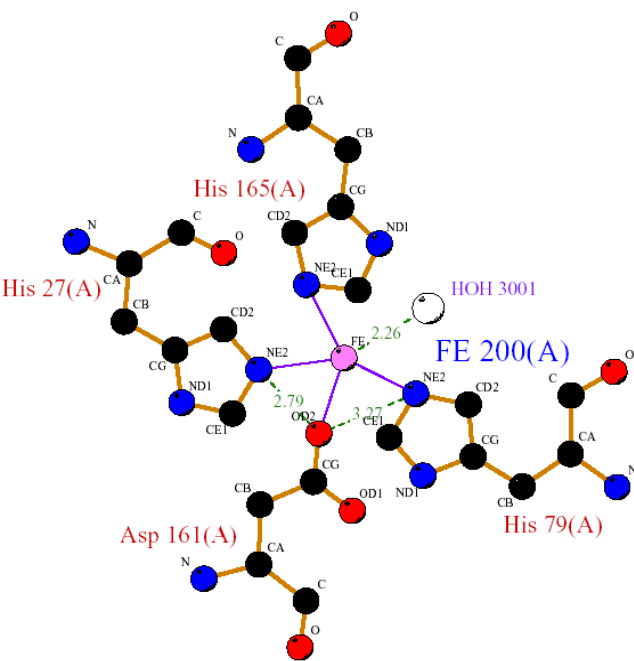


Figure 3
The active site residues of Fe Superoxide dismutase of *Thermosynechococcus elongatus*.

gen-bond network exerts a large influence on redox midpoint 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 *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].

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	F ₁₈₄ XXXA ₁₈₈ Q ₁₈₉T ₂₈₀F/Y ₃₀₃	R ₁₈₄ XXXG ₁₈₈ G ₁₈₉G ₂₈₀W ₃₀₃
Conserved residue with photosynthetic role	K87, K139	None
Transmembrane hydrophobic pocket	Absent	Present

* – Based on the structural analysis of MnSOD of *Anabaena* sp. (PDB No: 1gv3) and FeSOD of *Thermosynechococcus elongatus* BP-I (PDB No: 1my6)

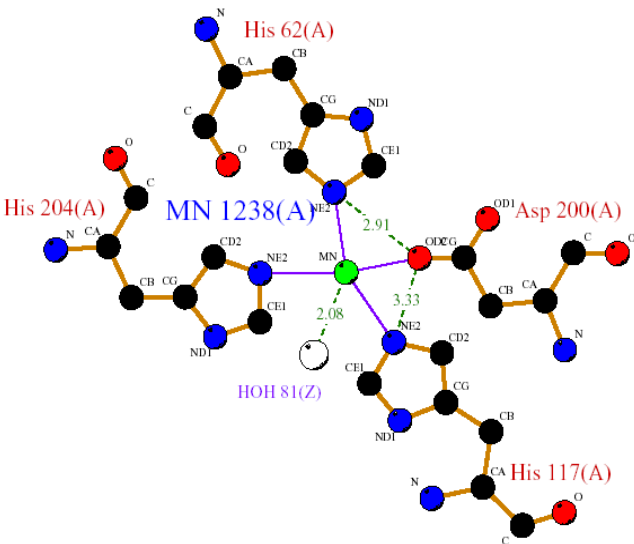


Figure 4
The active site residues of Mn Superoxide dismutase of *Anabaena* sp.

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 (*Synechococcus* sp. WH5701 (EAQ76095), *Synechococcus* sp. RS9917 (EAQ68777), *Trichodesmium erythraeum* IMS101 (EAO27349), *Anabaena variabilis* ATCC29413 (ABA21068) and *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 β-sheets (Fig 7A) accredited to be involved in confor-

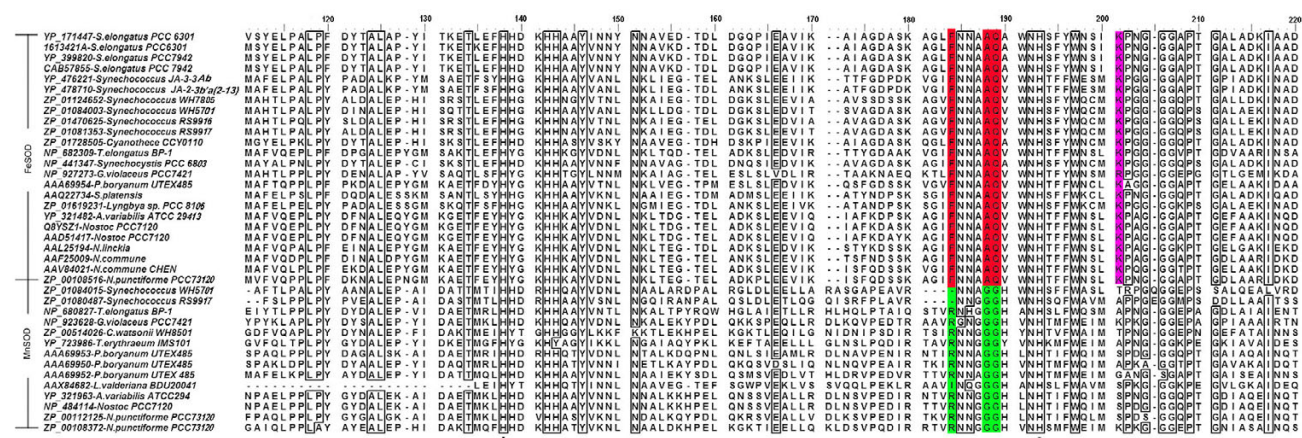


Figure 5

This figure shows the second quartile of protein sequence alignment of Fe and MnSODs in cyanobacteria. For full image, please see Additional file I. The conserved amino acid signature for Fe and MnSODs are highlighted in red and green respectively. Lysine residues of FeSOD involved in photosynthetic context is depicted in pink. The active site residues are labeled as I.

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 Cu²⁺

ion is by N81 of H103, N82 of H105, H147 and H215 and Zn²⁺ by N81 of three H147, 157, 171 and O81 of one D174 (Fig 8). Besides this, structural comparison designates the two specific hydrogen bonds between the Zn²⁺ coordinating residues D174-O81... H157-N81 (3.25 Å) and D174-O81... H171-N81 (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 heterotrophic forms lack this isoform (Table 2). The key for the ubiquity of NiSOD in *Prochlorococcus* may be due to

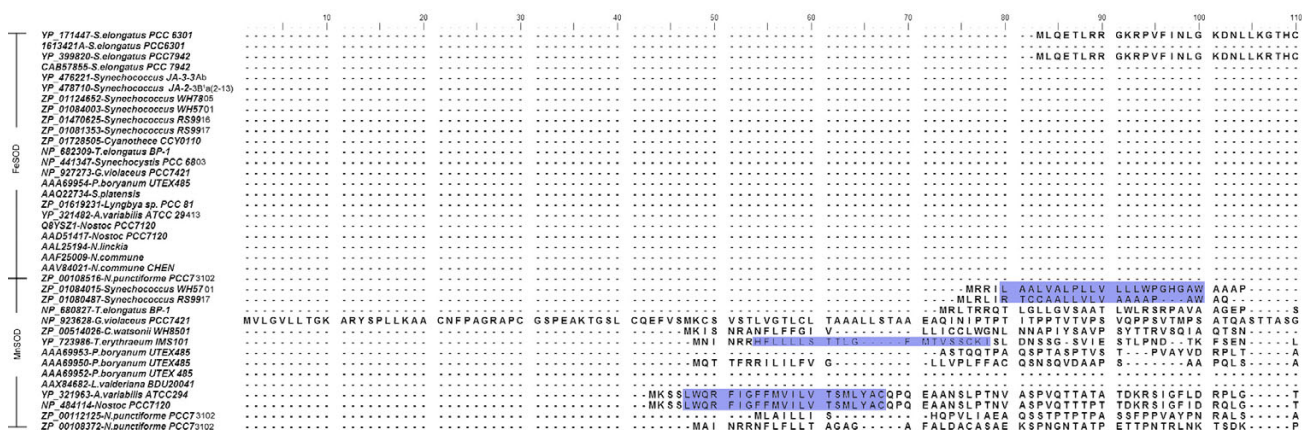


Figure 6

This figure shows the upper quartile of protein sequence alignment of Fe and MnSODs in cyanobacteria. For full image, please see Additional file I. Transmembrane hydrophobic pocket specific for membrane binding in MnSOD at the N-terminal region is highlighted in violet.

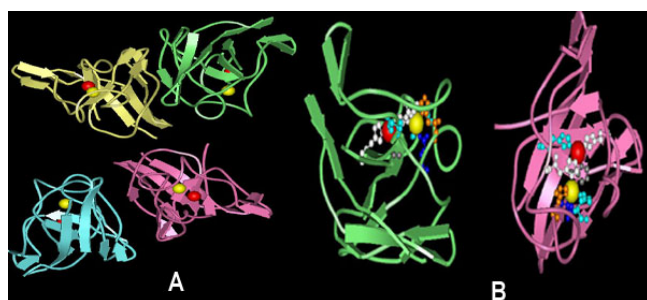


Figure 7
Representative structure of *Salmonella typhimurium* Cu/Zn superoxide dismutase. (a) Tetrameric subunits of Cu/ZnSOD. Chain A coded in green, B in pink, C in yellow and D in cyan. (b) Crystallographic structure of functional *S. typhimurium* Cu/ZnSOD (PDB 1eqw) subunit is represented to highlight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

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 (II) with an added axial His₁ 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

	10	20	30	40	50	60	70	80	90						
ZP_01472508-Synechococcus RS9916						QDLT	VKMTDLQ	..	TGKPVGTIEL	SNK.....	YGVVFIPE	33			
YP_001224674-SynechococcusWH7805						NTLT	VKMTDLSSG	..	TGENIGETIV	SETP.....	YGLLFTPH	36			
YP_381812-Synechococcus CC9605						QDLT	VKMTDLQ	..	TGKPVGTIEL	SNK.....	YGVVFIPE	33			
ZP_01468043-Synechococcus BL107						MRR-LIAL	MLVVSAVL	..	PGIAKAAVE	VTLHRISADG	VGESIGRVSA	RDSD.....	QGLEIIPS	59	
YP_376992-Synechococcus CC9902						MPR-LVPL	IALI-LLLCV	..	PGQASAGSL	VTLHAISAEG	VGDPIGTVKA	HDSD.....	QGLVITPS	58	
ZP_01123794-Synechococcus WH7803						MPRLVQL	VLLIGLITL	..	PGWCDA..LE	VPLQRIDANG	IGESIGSVTA	QDSD.....	QGLVIYPD	58	
ZP_01226581-Synechococcus RCC307	MSRRAQQMV	DDVTMKGPGP	NPMRL-LITI	TCLICWLIAA	P-IAKAEPIQ	ITMRSINADG	AGDVIGSVTA	..	KDSD.....	QGLVIFPD	80				
YP_730975-Synechococcus CC9311						MPR-LVPL	IALI-LLLFM	..	PGQASAGSL	VTVHSISAEG	VGAPIGSVQA	HDSD.....	QGLVITPT	58	
1B20-Escherichia coli						MRLLSLL	LFLSLLVPSM	..	P-ALAAEQQ	VWIQRIGGEE	SSEVLGSVMA	RDSD.....	DGLVISP	58	
1EQW-Salmonella typhimurium						MYR-LGAL	LALC-LALLM	..	PATVQASTIE	VTINSINTEG	IGESIGTISA	RDSD.....	QGLVIIPS	58	
11B5-Photobacterium leiognathi						NIPRKILSLL	FGLLLMVAIV	..	SCDQIGDSQS	TAEADNTEQI	VAVAEIKGAS	DSNLSGTITL	TENQPLDTSE	ILPTVEYKA	81
EAWS38062-Lyngbya PCC8106						HNAIVDRPGT	RWQGFVAFAG	..	IFGLLCLLLM	AVPAIDADRW	LQAKIELKNA	DGLPVGNASL	TQLS.....	DGVRVSVQ	77
NP_925116-G.violaceus PCC7412						MVCRSKVS	GFSLESSGCN	..	GSEEAASMSKH	LFLAGVGLW	LAAPALAGEQ	PAAVSAIKDL	NGQVVGTA	TF	81
NP_924927-G.violaceus PCC7412															81
	100	110	120	130	140	150	160	170	180						
ZP_01472508-Synechococcus RS9916	LADLTP-GMH	GFHIIHQNGSC	AS.....		SEK-DGKV	VLGGAAGGHY	DPEHTNKHGF	PWTDD.....	N HKGDLPALFV	97					
YP_001224674-SynechococcusWH7805	LNLTP-GIH	GFHVHTNPSC	MP.....		GMK-DGKE	VPALMAGGHL	DPEKTGKHLG	PYNDK.....	G HLGDLPLGVV	100					
YP_381812-Synechococcus CC9605	LADLTP-GMH	GFHIIHQNGSC	AS.....		SEK-DGKV	VLGGAAGGHY	DPEHTNKHGF	PYTD.....	N HKGDLPALFV	97					
ZP_01468043-Synechococcus BL107	LSGLTP-GEH	GFHLHANGSC	DS.....		AANADGAM	VAGLAAGGHW	DPDETGEHLG	PFGN.....	G HRGDL SRLVV	123					
YP_376992-Synechococcus CC9902	LQGLTP-GEH	GFHLHAGDSC	DA.....		ALNAEGVM	VAGLAAGGHW	DPDETNTHLG	PFGN.....	G HRGDL SRLVV	122					
ZP_01123794-Synechococcus WH7803	LADLTP-GEH	GFHLHSTGSC	EA.....		GQTAAQGT	IAGLAAGGHW	DPDETGHGLG	PFGN.....	G HRGDL SRLVV	122					
ZP_01226581-Synechococcus RCC307	LANLTP-GDH	GFHVHNSPSC	EA.....		ALNPEGES	VAGLAAGGHW	DPDNTSGTHLG	PFGS.....	G HRGDL SKLIV	144					
YP_730975-Synechococcus CC9311	LNLTP-GDH	GFHLHNSPSC	EA.....		ALNPEGES	VAGLAAGGHW	DPDNTSGTHLG	PFGS.....	G HRGDL SKLIV	144					
1B20-Escherichia coli	LQGLTP-GEH	GFHLHAGDSC	DA.....		AMNGEGEM	VAGLAAGGHW	DPDETNTHLG	PFGN.....	G HRGDL SKLIV	122					
1EQW-Salmonella typhimurium	ISGLAA-GAY	GFHLHENGSC	ES.....		GLK-DGVE	VAGLAAGGHW	DPDNTSGHQH	PFGV.....	G HRGDL SRLVI	121					
11B5-Photobacterium leiognathi	LSGLTP-GEH	GFHLHAGDSC	AP.....		QTNSEGAS	IAGLAALGHW	DPDQNTNLHG	PFGN.....	G HRGDL SRLVV	122					
EAWS38062-Lyngbya PCC8106	VSGLPPNTHK	GFHIIHQTAAC	EP.....		DFG.....	AAGGHF	DGPYGETNP	DANHP.....	F HMGDLPLNVA	138					
NP_925116-G.violaceus PCC7412	VQGLLP-GKY	PIHFHSGKGC	VAP.....		DFRSSRGVF	DTHSLGHKTR	PDGQP.....	VP PAGLLPALIV	135						
NP_924927-G.violaceus PCC7412	VQGLQP-GKH	AMHIIHAGVKC	EPPAFKSAGP	HLGDAKMAPT	SGTPHSHGAK	GATALSMNHA	HSAGSMASSI	PHNHGAMHAA	MAGDLPDLVV	170					
	190	200	210	220	230	240									
ZP_01472508-Synechococcus RS9916	SANGLATNPV	LAPRLT.....	..LKEKLGH	AIMIHAGGDN	HSDMPK..AL	GGGGARVACG	VIQ...	151							
YP_001224674-SynechococcusWH7805	NADGTATYPL	LAPRLK.....	..LSELKGH	SLMIHKGDN	YSDKPA..PL	GGGGARFACG	VIEK...	156							
YP_381812-Synechococcus CC9605	SANGLATNPV	LAPRLT.....	..LKEKLGH	AIMIHAGGDN	HSDMPK..AL	GGGGARVACG	VIQ...	151							
ZP_01468043-Synechococcus BL107	NDGTTPTSV	VAPRLS.....	..TADLRGK	ALVVHAGGDT	YSDTP...AL	GGGGARVACG	VVGR...	177							
YP_376992-Synechococcus CC9902	DADGNTTTT	VAPRLS.....	..TADLRGR	ALVVHAGGDT	YSDVP...PL	GGGGARVACG	VG...	174							
ZP_01123794-Synechococcus WH7803	DDGKTNTSV	VAPRLS.....	..TADLRGK	ALVVHAGGDT	YRDEP...PL	GGGGARVACG	VVPDER	178							
ZP_01226581-Synechococcus RCC307	DEDGTTKTSV	VAPRLN.....	..TKALKGR	ALIVHAGGDT	YSDTP...PL	GGGGERVACG	VINS...	198							
YP_730975-Synechococcus CC9311	DADASTKTSV	VAPRLI.....	..TNDLRGK	ALVVHAGGDT	YSDTP...AL	GGGGARVACG	VVN...	175							
1B20-Escherichia coli	NADGNTTTT	VAPRLS.....	..TADLRGR	ALVVHAGGDT	YSDIP...PL	GGGGARVACG	VG...	174							
1EQW-Salmonella typhimurium	NADGTTTTPV	VAPRLS.....	..VDDLKGR	AFIVHAGGDT	YSDPE...PL	GGGGARVACG	VAGE...	175							
11B5-Photobacterium leiognathi	DRDGNNTTSV	VAPRLK.....	..ASDLRGR	ALVVHAGGDT	YSDTP...PL	GGGGARVACG	VGS...	175							
EAWS38062-Lyngbya PCC8106	DAQGNVFTH	RTSRVTLSSG	PLSLFDEDS	AFIVHIDED	QGTGTG..QG	GAGGGRLGCG	IEKQA	201							
NP_925116-G.violaceus PCC7412	GAAGTGELNA	LNTDVT.....	..LRAHKLH	SLLRPGGSAL	VIIHAAHSRQI	IACGAVTRTP	VSD...	191							
NP_924927-G.violaceus PCC7412	GSDGSGKAEI	LNAEVRITPG	RNFLQSEGT	ALLIHDVADP	NQRIAC..GV	ISKAPNSSPE	AKVNP...	233							

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
<i>Prochlorococcus marinus</i> AS9601	YP_001009883	157	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> CCMP1986	NP_893411	156	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> CCMP1375	NP_875759	157	Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9301	YP_00109170	157	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9303	YP_001017980	164	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9211	ZP_01004940	140	Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9312	YP_397886	157	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9313	NP_894173	157	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> MIT 9515	YP_001011769	157	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> NATL1A	YP_0010155334	163	putative Ni	NiSOD
<i>Prochlorococcus marinus</i> NATL2A	YP_292055	163	putative Ni	NiSOD
<i>Synechococcus</i> sp. WH 8102	NP_897719	157	putative Ni	NiSOD
<i>Synechococcus</i> sp. BL107	ZP_01469600	157	putative Ni	NiSOD
	ZP_01468043	198	putative SOD	Cu/ZnSOD
<i>Synechococcus</i> sp. CC9605	YP_381196	157	putative Ni	NiSOD
	YP_381812	178	SOD precursor (Cu-Zn)	Cu/ZnSOD
<i>Synechococcus</i> sp. CC9311	YP_729969	175	Cu/Zn	Cu/ZnSOD
	YP_730975	155	Ni	NiSOD
<i>Synechococcus</i> sp. CC9902	YP_376992	175	putative SOD	Cu/ZnSOD
<i>Crocospaera watsonii</i> WH 8501	ZP_00517273	159	Hypothetical protein	NiSOD
	ZP_00514026	254	SOD	MnSOD
<i>Synechococcus elongatus</i> PCC 6301	YP_171447	229	SOD	FeSOD
	1613421A	202	SOD	FeSOD
<i>Synechococcus elongatus</i> PCC 7942	YP_399820	229	SOD	FeSOD
	CAB57855	201	SOD	FeSOD
<i>Synechococcus</i> sp. JA-3-3Ab	YP_476221	199	Fe	FeSOD
<i>Synechococcus</i> sp. JA-2-3B'a(2-13)	YP_478710	199	Fe	FeSOD
<i>Synechococcus</i> sp. WH 7805	ZP_01124652	199	SOD	FeSOD
	ZP_01123794	174	putative SOD	Cu/ZnSOD
<i>Synechococcus</i> sp. WH 5701	ZP_01084003	199	SOD	FeSOD
	ZP_01084015	231	Mn	MnSOD
<i>Synechococcus</i> sp. RS9916	ZP_01470625	199	SOD	FeSOD
	ZP_01472508	177	SOD precursor (Cu-Zn)	Cu/ZnSOD
<i>Gloeobacter violaceus</i> PCC 7421	NP_927273	203	SOD	FeSOD
	NP_923628	316	SOD	MnSOD
	NP_924927	233	similar to SOD	NA*
	NP_925116	191	similar to SOD	NA*
<i>Synechococcus</i> sp. RS9917	ZP_01081353	199	SOD	FeSOD
	ZP_01080487	229	SOD	MnSOD
<i>Cyanothece</i> sp. CCY0110	ZP_01728505	200	SOD	FeSOD
<i>Thermosynechococcus elongatus</i> BP-1	NP_682309	200	SOD	FeSOD
	NP_680827	240	SOD	MnSOD
<i>Lyngbya</i> sp. PCC8106	ZP_0169885	201	SOD	Cu/ZnSOD
	ZP_01619231	201	SOD	FeSOD
<i>Trichodesmium erythraeum</i> IMS101	YP_723986	254	SOD	MnSOD
	YP_720765	159	putative Ni	NiSOD
<i>Synechocystis</i> sp. PCC 6803	NP_441347	199	Fe	FeSOD
<i>Spirulina platensis</i>	AAQ22734	170	Fe	FeSOD
<i>Plectonema boryanum</i> UTEX 485	AAA69954	199	Fe	FeSOD
	AAA69953	239	superoxide dismutase [Mn] precursor	MnSOD
	AAA69950	248		MnSOD
	AAA69952	206		MnSOD
<i>Leptolyngbya valderiana</i> BDU20041	AAX84682	144	Mn	MnSOD
<i>Nostoc punctiforme</i> PCC 73102	ZP_00108516	200	SOD	FeSOD
	ZP_00112125	249	SOD	MnSOD
	ZP_00108372	259	SOD	MnSOD
<i>Nostoc</i> sp. PCC 7120	Q8YSZ1	200	Fe	FeSOD
	AAD51417	200	Fe	FeSOD

Table 2: Annotation of cyanobacterial superoxide dismutases based on sequence and structure conservation. (Continued)

<i>Anabaena variabilis</i> ATCC 29413	NP_484114	270	SOD	MnSOD
	YP_321482	200	Mn/Fe	FeSOD
	YP_321963	270	Mn/Fe	MnSOD
<i>Nostoc linckia</i>	AA125194	200	SOD	FeSOD
<i>Nostoc commune</i>	AAF25009	200	SOD	FeSOD
<i>Nostoc commune</i> CHEN	AAV84021	200	Fe	FeSOD

* Not Assignable (NA)

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 possess 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).

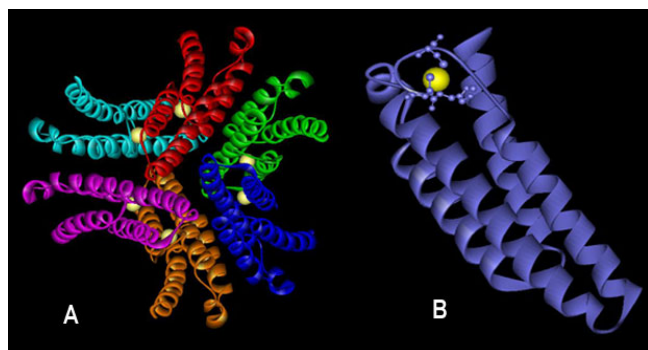


Figure 9
Schematic view of representative NiSOD subunit and hexameric structure of *Streptomyces coelicolor* [PDB 1t6u]. (a) NiSOD biological unit is a hexameric assembly of 4-helix bundles (b) NiSOD subunit with metal binding hook labels at the end of helix-I along with the metal shaded in yellow is represented by ball and stick mode as visualized in WebLab ViewerLite 4.2 software.

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 nnPREDICT [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. LU 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 amino acid 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 *.*

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Acknowledgements

This study was supported by Department of Biotechnology, Government of India, New Delhi. The authors also thank Dr. Kaeel Ahmad, Reader, Jamal Mohammed College, Tiruchirappalli, India for his critical comments and valuable suggestions.

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