Detection of a gene for iron containing superoxide dismutase in the terrestrial cyanobacterium
Tolypothrix byssoidea

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Using oligonucleotide probes deduced from a conserved region of amino acids of SOD (superoxide dismutase) proteins, one SOD gene was detected in the cyanobacterium, Tolypothrix byssoidea, isolated from the exposed rock surface of Sun temple, Konark. Amplification of specific genes of the genomic DNA and sequencing of the PCR product yielded a sodB gene of 292 bp size. Southern hybridization confirmed the presence of a single sodB gene in T. byssoidea.

Keywords: FeSOD, sodB gene, southern analysis, cyanobacteria, Tolypothrix byssoidea, terrestrial habitat

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Superoxide dismutases (SODs, EC 1.15.1.1), found in all aerobic organisms, protects cells by dismutating superoxide radicals to hydrogen peroxide and molecular oxygen. Four types of SODs are known based on the metal cofactor at the active site: iron (Fe-SOD), manganese (Mn-SOD), copper/zinc SOD (CuZn-SOD), and nickel (Ni-SOD). In prokaryotes including cyanobacteria, only Fe- and Mn-SODs have been detected1-5. These reports as well as genome analysis of different cyanobacteria showed that these organisms contain either one or more types of SOD genes6-8. Localization experiments and analysis of sequence revealed that sodB encoding Fe-SOD was cytosolic, while sodA encoding Mn-SOD was a membrane bound homodimeric protein9. Moreover, no Mn-SOD was observed in the isolated heterocyst9 suggesting that two different SOD proteins have differential roles in this organism. In addition, a soluble 21 kDa Fe-SOD polypeptide was reported in the cyanobacterium, Nostoc commune CHEN, which was released upon hydration of prolonged desiccated cells (after 13 years of storage). Based on the N-terminal sequence of this protein one sodF gene was detected, which countered the effects of oxidative stress during desiccation and rehydration10. The cyanobacterium, Tolypothrix byssoidea, occurring on the exposed rock surfaces of temples and monuments in various regions of India has been reported to exhibit marked tolerance to higher solar radiation coupled with desiccation stress11. Presence of Fe-SOD was demonstrated in the cyanobacterium inhabiting the crust as well as culture in native gels12. The quantity of SOD did not change even after storage of the crust in desiccated state. Hence, the presence of the sodB gene was investigated in this organism. Hybridization of genomic DNA with the PCR product was also carried out for confirmation.

Axenic culture of T. byssoidea (Agardh ex. Bornet & Flahault) Kirchner UU53170, isolated from the crust forming on Sun temple, Konark in the Orissa state of India13, was used in the experiments. The organism was grown in nitrogen free BG11 medium12 at 25°C under 7.5 W.m² light intensity. Exponentially growing cells were pelleted by centrifugation. The cell pellet was resuspended in 567 µL lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM trisodium citrate and 1.5% SDS) and incubated for 1 h at 37°C, after addition of 5 mg of lysozyme, 30 µL of 10% SDS and 5 mg proteinaseK. To this 100 µL 5 M NaCl was added, followed by addition of 80 µL CTAB/NaCl solution (10% CTAB/0.7 M NaCl) and incubated for 10 min at 65°C. The lysate was extracted with equal volume of chloroform:isoamyl alcohol (24:1), followed by equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The DNA was precipitated with 0.6 volume isopropyl alcohol, followed by washing with 20% ethanol and dried in a Speed Vac. On the basis of codon usage data from existing cyanobacterial sodB and sodF genes, a set of degenerate oligonucleotide primers (5’TGA TAA AAG CTG GGC GAG CA–3’ and 5’–ACA GTC TCA CCG ATG GTA CA–3’) was obtained from ROTH (Carl Roth GmbH, Germany) and used to amplify the sodB gene from the genomic DNA of T. byssoidea. Amplification was done in a...
thermocycler under the following conditions: denaturation at 95°C for 5 min, followed by 30 cycles of amplification with denaturation at 95°C for 45 sec, annealing at 61°C for 40 sec and extension at 72°C for 1 min. An extension step at 72°C for 5 min was added after completion of 30 cycles. The amplified product was separated in a 1.5% agarose gel, followed by purification using Qia quick gel extraction kit. DNA sequencing of the PCR product was performed in an ABI 373A (P/N 402079) DNA sequencer. For southern hybridization, DNA extracted from *T. byssoidea* was digested with *Bam*HI, *Hpa*I, *Pst*I and *Sac*I, and transferred to nitrocellulose membrane (Biodyne® B) following Sambrook *et al.*

13. The immobilized genomic DNA was hybridized with the PCR product labeled with [α-32P] dcTP (specific activity, 2700 ci/m mol) and labeled bands were visualized by phosphoimaging (Biorad).

By using oligonucleotide probes constructed from a conserved region of amino acids of SOD proteins, a purified PCR product of 292 bp was obtained from the genomic DNA of *T. byssoidea* as of the putative *sodB* gene. Fig. 1 depicts the nucleotide sequence of the gene and the deduced amino acid sequence. It shows a 292 bp open reading frame coding for a polypeptide of 96 amino acids. The resulting sequence showed 49.2% identity to the *sodB* gene of *Anabaena* sp. PCC 7120. The derived Fe-SOD amino acid sequence of *T. byssoidea*, when aligned with Fe-SOD amino acid sequence of *Anabaena* sp. PCC 7120, also showed 32.1% identity (Fig. 2). For detection of the gene in the organism, genomic DNA was digested with several restriction enzymes prior to membrane transfer and hybridization with the PCR amplified *sodB* gene, and then pattern of hybridization was analyzed (Fig. 3). Distinct bands were detected in the restriction enzyme digests, suggesting the presence of a single *sodB* gene in the genome of the cyanobacterium. *T. byssoidea* colonises the exposed rock surfaces of monuments under tropical climatic conditions of India and survives the hot summer months11. Under such intense solar radiation, organisms are prone to desiccation damage as they evolve intracellular oxygen through photosynthesis. To counter such stress, the cyanobacterium possibly uses Fe-SOD to scavenge the superoxide radicals and survives under such high solar insolation.

Since *T. byssoidea* is a desiccation tolerant organism, it might also possess *sodF* encoding for Fe-SOD during rehydration as that was detected in *Nostoc commune* CHEN10. Using a different set of oligonucleotide probes constructed for amplification of the *sodF* gene (5′–AAT TCT TGA ACG CCG GA–3′ and 5′–ACA AGC TCA CTG AAG GTA CA–3′), another PCR product was also obtained from the genomic DNA of *T. byssoidea*. Sequence analysis showed that it was a 358 bp product, but contained several interruptions in the sequence (data not shown). However,

![Nucleotide sequence of 292 bp PCR product of *T. byssoidea* and the deduced amino acid sequence.](image)

Fig. 1—Nucleotide sequence of 292 bp PCR product of *T. byssoidea* and the deduced amino acid sequence.
since the organism lives under extreme arid environments and exposed to multiple cycles of desiccation and rehydration, it might be containing a gene similar or different to that sodF encoding for soluble Fe-SOD proteins as was found in \textit{Nostoc commune} CHEN, isolated from an entirely different terrestrial habitat\(^{10}\).

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References


