

ABSTRACT

Anthropogenic activities are dumping heavy metals into the environment as waste effluents or integral part of some compounds. This has resulted in an increase in metals concentration, more than the required threshold, leading to metal toxicity for aquatic life. Metal resistant ciliates remove metal ions from contaminated water, mainly by the process of bioaccumulation. This bioaccumulation is due to low molecular weight, metal ions chelating proteins known as metallothioneins.

In the present study, a new species of *Tetrahymena* (*Tetrahymena*1.7) is being reported from the local industrial wastewater. Analysis of *Tetrahymena*1.7 SS rDNA (accession# HE820726) showed 99% homology to seven different species of the genus *Tetrahymena*. SS rRNA secondary structure appeared in 40 helices with 18 variations, including 17 substitutions and one deletion. All the variations are present in 6 variable lengths, namely, V2, V3, V4, V7, V8 and V9. Cytochrome c oxidase subunit 1 (COX1) gene sequence was quite variable, with 91% homology to its closest relative *T. thermophila*. Since this value was higher than the intraspecific variations ($\geq 99\%$ homology), *Tetrahymena*1.7 has been considered as a new species i.e., *Tetrahymena farahensis*. Phylogenetic analysis based on both SS rDNA and COX1 using maximum likelihood and neighbor joining methods showed that *Tetrahymena farahensis*, new species was related to *T. thermophila* and *T. malaccensis*. Thus, it appeared to be a new member of riboset A1 and coxiset A1 on the basis of SS rRNA and COX1 gene, respectively.

Tetrahymena farahensis showed best growth rate in copper supplemented Neff's medium. However, its growth was two fold in Bold-basal salt medium compared to copper supplemented Neff's medium and modified Neff's medium. The organisms showed best growth at $27 \pm 1^\circ\text{C}$ and pH 7.0 to 7.5.

Tetrahymena farahensis could tolerate $127\mu\text{M}$, $143\mu\text{M}$ and $1270\mu\text{M}$ of copper in wheat grain medium, bold basal salt medium and modified Neff's medium, respectively. The organisms showed bimodal uptake of copper, first in 15-30 min and the second after 5h at low ($78.5\mu\text{M}$ and $157\mu\text{M}$) copper exposure. At higher ($786\mu\text{M}$ and $1573\mu\text{M}$) copper concentration, the first uptake was shifted to 0-15min while timing for the second

uptake remained the same (5h). After 96h, the organisms showed 54.9% copper uptake at 500 μ M copper stress.

A new copper metallothionein gene has been identified from *Tetrahymena farahensis*. The nucleotide sequence of *T. farahensis* copper metallothionein (*TfCuMT*) was submitted to gene bank under accession no. HE820725. BLAST results of *TfCuMT* showed 83% homology with already reported copper metallothioneins. The gene size is 327pb, encoding 108 amino acids peptide chain. Like most of the metallothioneins cysteine residues contribute nearly 30%. In *TfCuMT*, TAA and TAG encode glutamine as already reported for ciliates genetic code. The peptide sequence has copper metallothionein characteristic CXC motifs and devoid of any cadmium metallothionein specific CCC motif. Structural repeats present in peptide sequence of *TfCuMT* indicate internal duplication of gene at some stage of gene evolution. The theoretical PI is slightly basic, a characteristic of most of metallothioneins. The predicted irregular structure of *TfCuMT* shows that it is functional in the presence of some metal ions. Functional analysis using metalmine software showed that most of the cysteine residues are involved in copper binding.

For expression of *TfCuMT* in *E.coli* host cells, TAA and TAG were mutated to CAA and CAG using site specific mutagenic primers and mega primer. The mutated gene showed higher expression in pET28a expression vector compared with pET21a. Optimum expression was obtained after 6-8h of 0.1mM IPTG induction. Stability of His tagged *TfCuMT* in 5% SDS was low, with roughly about 100 min half-life. Presence of 1.0 μ M copper increased the expression level. Expression of *TfCuMT* is directly proportional to the addition of cysteine in the growth medium. His tagged *TfCuMT* was purified through affinity chromatography using NTN-His binding resin in the presence of 0.1M imidazole and NaCl.

Real time PCR based quantitative analysis showed that *TfCuMT* was a copper inducible gene. Gene had a basic expression level which increased by the induction of copper ions. Maximum expression was observed within 15min of copper exposure.