Abstract

Almost all the eubacterial groups of microbes have developed resistance mechanisms against almost all of the toxic metals, in response to the selective pressures from the environment loaded with metals. Copper is an essential metal having relative abundance approximately 68 ppm in the earth crust and is required by the microbes and other organisms for their different structural and catalytic properties. Differentiation and metabolism of cell is affected by toxicity caused by Cu at almost every level of cellular life. Cu toxicity is based upon hydroperoxide radical's formation and further its interaction with cell membrane. In *Klebsiella pneumoniae* KW, different resistance mechanisms are working for the copper homeostasis and different genes are regulating these mechanisms. CueR and CueO are MerR-like transcription activator protein and multicopper oxidase, respectively. Both proteins are responsible of maintenance of Cu metabolism. Both *cueR* and *cueO* were amplified and cloned in pTZ57R/T by using TA cloning procedure, successful cloning was confirmed by double restriction analysis and DNA sequencing technique. Sequence analysis of *cueR* was done using different online available tools to study its structural and functional properties. *cueR* was further cloned in pTZ57R/T vector by introduction of restriction site of *Nde* I enzyme. Positive clones were subjected to the expression analysis using pET21a(+) expression vector. Expression was taken in different conditions and was properly optimized. Double restriction analysis of both the genes in first cloning gave exact fragments of 478 bp and 1.653 kb for *cueR* and *cueO,* respectively along with the band of size 2.8 kb cloning vector. Sequence analysis of *cueR* and CueR from *Klebsiella pneumonia* KW provided its maximum sequence similarity within the same genus. No significantly homologous protein was found in other orders or at higher systematic level. Multiple alignment revealed that the homology increased at protein level as compared to neucleotide level in all bacterial strains belonging to the family Enterobacteriaceae. Sequence analysis further revealed the cytosolic and soluble nature of CueR with no membrane spaning regions, horn like homodimeric functional form having alpha helices and beta sheets, three conserved sites in each monomer including dimer interface, DNA binding site and copper binding site. Recombinant DNA pTZ57R-CueR (along with *Nde* I restriction site) was confirmed by getting two bands on gel one for *cueR* (457 bp) and other for pTZ57R/T vector (2.8 kb). Recombinant pET21a-cueR$_{Exp}$, upon restriction digestion showed two band of sizes 457 bp and 5.4 kb for cueR and pET21a (+) vector, respectively. Expression of CueR induced with 0.05 mM IPTG for 6 hours was taken at 37 °C which gave a 13 kDa protein band in supernatant that was accurately the size of the respective protein showing its soluble nature. Optimal conditions for the best expression were found to be 37 °C with 0.02 mM IPTG induction for 4 hours.