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Over-expression of the multicopy-associated filamentation (*maf*) gene of *Caldimonas manganoxidans* MS1 and determination of the biological effect of MAF proteins on the viability of bacterial cells

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Multicopy-Associated Filamentation (MAF) proteins represent a large family of conserved proteins. MAF protein is a nucleic acid binding, intra-cellular protein. The exact biochemical activity and functional mechanism of MAF protein remain unknown. However, it is believed that MAF protein has an inhibitory effect on septation, DNA and RNA synthesis and an intrinsic house cleaning function. *Caldimonas manganoxidans* is a Gram negative, thermophilic organism. The proteins produced by thermophilic microorganisms are known to have thermostable properties and high stability under extreme conditions, hence they can be considered as potential candidates for many industrial applications. The objective of this research was to clone the *maf* gene of *C. manganoxidans* MS1, express and determine the biological effect of recombinant MAF protein on cell viability of *C. manganoxidans* MS1, a native thermophilic organism previously isolated from Maha Oya hot water springs in Sri Lanka. The genomic DNA was extracted from the organism and the complete *maf* gene of *C. manganoxidans* MS1 was PCR amplified using gene specific primers, and initially cloned into pGEM®-T plasmid vector and transformed into the cloning host, *Escherichia coli* JM 109. Thereafter, the *maf* gene was cloned into the expression vector, pET 28a(+) plasmid and transformed into *E. coli* BL21 (DE3) pLysS expression host. Recombinant colonies were confirmed by colony PCR technique. The over-expressed MAF protein was purified from culture by using MagneHis™ protein Purification System. SDS-PAGE analysis indicated a molecular weight of around 22 kDa for the recombinant MAF protein and a concentration of approximately less than 0.2 μg/μL. Nucleotide BLAST (NCBI) of the complete nucleotide sequence obtained for *C. manganoxidans* MS1 *maf* gene from *E.coli* BL 21 (DE3) pLysS showed 99% identity with the complete sequence of *maf* gene (Accession: WP_026329982.1, GI: 648638231) of *C. manganoxidans* ATCC BAA-369 (Accession: NZ_KB905929.1, GI: 485071406). *C. manganoxidans* MS1 cells were exposed to the over-expressed, purified, recombinant MAF protein to determine the biological effect of MAF protein on cell viability. The bacterial cell viability was assessed by plate count method. Upon exposure to MAF protein, there was a remarkable decrease in CFU/mL with time, whereas there was a remarkable increase of CFU/mL with time when the bacterial cells were not exposed to MAF protein. In conclusion, *maf* gene from native *C. manganoxidans* MS1 strain was successfully cloned, expressed and MAF protein was purified from *E.coli*. The recombinant MAF protein has a negative effect on cell viability of *C. manganoxidans* MS1 strain, itself.

Keywords: MAF proteins, *Caldimonas manganoxidans*, Cloning