Expression and functional characterization of recombinant metallothionein proteins of water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichhornia crassipes*) in *E. coli*

Water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichhornia crassipes*) are aquatic plants native to South America and are known for their ability to hyperaccumulate heavy metals from water without showing any symptoms of abiotic stress. This is achieved through several mechanisms including phytoextraction. During phytoextraction, the production of peptides, such as metallothioneins, has been shown to improve the plant's tolerance to and accumulation of heavy metals.

Figure 1: *P. stratiotes* (A) and *E. crassipes* (B)

Metallothioneins (MTs) are gene-encoded polypeptides that have highly conserved cysteine-rich motifs contributing close to 30% of the polypeptide chain. Studies have shown that MTs participate in various heavy metal tolerance processes in plants because of their capability of chelating and sequestering heavy metal ions such as zinc, copper, and cadmium using thiol groups present in cysteine residues.

In our study, novel putative MT genes from *P. stratiotes and E. crassipes* were identified using bioinformatics and PCR, and gene expression analysis showed upregulation of proposed MTs from *P. stratiotes* exposed to copper. To further improve our understanding of the function of novel putative MT genes from *P. stratiotes* and *E. crassipes,* we focused on optimizing recombinant MT protein expression in *E. coli* and investigating functions of MTs by means of increase in tolerance and accumulation of copper by transgenic *E. coli* harbouring putative novel MTs.

Optimum conditions for recombinant soluble MT protein production in *E. coli* were observed, and an increase in tolerance and accumulation of copper by transgenic *E. coli* was confirmed. Characterizing of novel MT sequences is crucial for eco-engineers aiming to effectively utilize *P. stratiotes* and *E. crassipes* for alleviating heavy metal contamination in water.

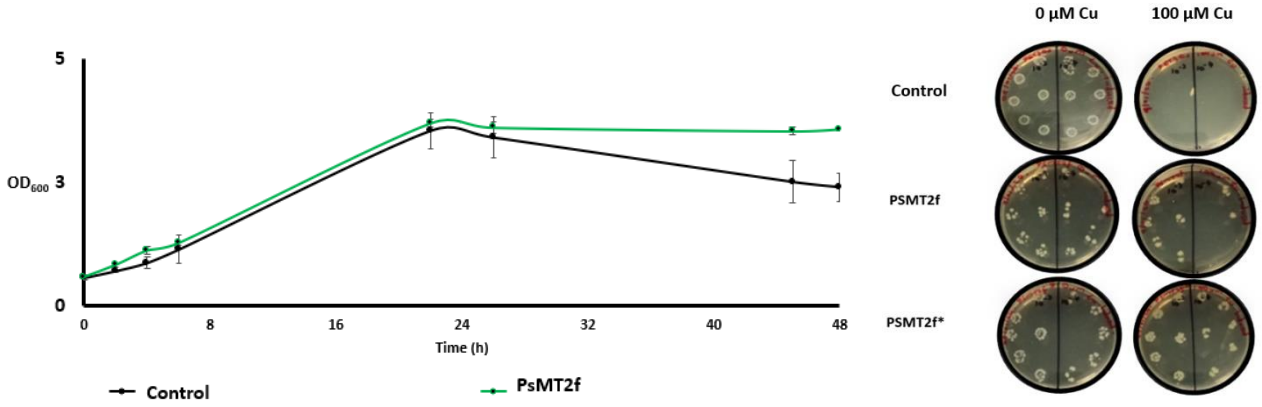

Figure 2: Growth of study clones in the presence of 100 µM Cu