The human pathogen *Clostridium difficile*: a look at a putative glyoxalase involved in protection from oxidative stress

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**Background:** Hospital acquired infections due to *Clostridium difficile* (*C. diff*) is associated with nosocomial diarrhea and pseudomembranous colitis. Using a published microarray analysis of *C. diff* strain 630, several open reading frames (ORFs) were noted for their upregulation under different environmental stresses. One of these genes (CD1134) is a putative glyoxalase I. The glyoxalase enzymes detoxify a side product of glycolysis, methylglyoxal, and use metals as a cofactor.

**Objectives:** To characterize a putative glyoxalase I protein from *Clostridium difficile* 630.

**Methods:** Using bioinformatic analysis we compared known glyoxalase genes from other species with the putative glyoxalase I of *C. diff*. The ORF CD1134 was cloned into an expression vector and the protein was overexpressed in *Escherichia coli* and purified. Mass spectrometry (MS) will be used to determine the molecular weight through observing the mass to charge ratio. Inductively Coupled Plasma MS was used to determine the metals bound to the as-purified protein. A metal activation study was conducted with several metal chlorides to observe the effect each metal has on enzyme activity. The recombinant enzyme is currently being further characterized for optimal pH and substrate specificity.

**Results:** Through bioinformatic analysis it was observed that putative glyoxalase I CD1134 shared the same conserved metal binding domain as that found in GlxI of *Escherichia coli* and *Pseudomonas aeruginosa*. Based on other studies of glyoxalase I both *E. coli* and *P. aeruginosa* proteins are nickel activated enzymes. Through our studies we found that the putative glyoxalase CD1134 of *C. difficile* 630 does have glyoxalase activity *in vitro* and appears also to be a nickel activated protein.