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## Interplay between expression of sulfur assimilation pathway genes and metal (Cd, Zn, Pb) stress in *Acidithiobacillus ferrooxidans*

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*Acidithiobacillus ferrooxidans* plays a critical role in metal solubilization in the biomining industry, and occupies an ecological niche characterized by high acidity and high concentrations of toxic heavy metal ions. In this paper, we investigated the interplay between metal resistance, SAP gene expression and thiol-containing metabolite levels. Cells grown in the presence of metal (Cd, Zn, Pb) had effected activities for the following enzymes: adenosyl phosphosulfate reductase (APR), serine acetyl transferase (SAT) and O-acetylserine (thiol) lyase (OAS-T). We investigated the concentrations of mRNA transcripts of the genes encoding these enzymes in cells grown in the presence of metal transcripts for 4 SAP genes- ATPS (ATP sulfurylase), APR, SiR (sulfite reductase), SAT and OAS-TL; each showed more than three-fold concentration increase. At the metabolite level, concentrations of intracellular cysteine and glutathione (GSH) were nearly doubled. These results suggested that Cd and Zn induced SAP pathway gene transcription, while Pb inhibited SAP gene expression and enzyme activities compared to the pathway in most organisms. Since the detoxification function of thiol pool, the results also suggested that the high resistance of *A. ferrooxidans* to Cd, Zn may also be due to regulation of GSH and the cysteine synthesis pathway.

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## Chitosan-propolis nanoformulation for combating *Enterococcus faecalis* biofilms *in vitro*

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*Enterococcus faecalis* are bacteria commonly detected in asymptomatic, persistent endodontic infections that grow in the presence or absence of oxygen. They cause urinary tract infections, wound infections, bacteremia, endocarditis, endodontic infections and are also capable of forming biofilms in implant devices. Propolis is a resinous substance rich in flavanoids and has anti-bacterial properties. Malaysian propolis was obtained from the bee farms and tested for its effect on biofilm formation by *E. faecalis in vitro*. A 20% extract of propolis was prepared using ethanol or ethyl acetate. Chitosan-propolis nanoparticles were prepared by ionotropic gelation of chitosan with tripolyphosphate of sodium. Chromatographic analysis was performed by using HPLC. The nanoparticles were characterized in terms of average particle size, polydispersity index, zeta-potential and morphological characteristics. The average particle size in the nanoformulation measured by transmission electron microscopy was 125-200 nm. The zeta potential calculated ranged between 33-37±6 mV depicting good stability. *E. faecalis* was allowed to form biofilms in 96-well microtiter plates (Nunc) and the efficacy of the different extracts of propolis as well as the nanonformulation in inhibiting the biofilms was tested. Biofilm growth was monitored and bacterial viability in the biofilm was calculated. Nanoformulation of propolis gave the best inhibitory effect (at 75 µg) compared to ethanol and ethyl acetate extracts (200 µg). The effect of the nanoformulation on the expression of bacterial genes involved in biofilm formation was also studied. Sustained release by biodegradable chitosan flavonoids nanoformulation is able to provide long term disinfection leading to effective therapy.

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