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"Cellular mechanisms of selenium resistance in *Stenotrophomonas bentonitica* BII-R7: Flow cytometry, microscopic and spectroscopic characterisation"

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Selenium (Se) is an essential metalloid with important biotechnological applications and biological functions as a trace element for living organisms. Se exists in different valence states: selenate [Se(VI)], selenite [Se(IV)], elemental selenium [Se(0)] and selenide [Se(-II)]. The solubility and mobility of this element mainly depends on its oxidation state. Basically, the oxidised forms are the most toxic due to their high solubility. Globally, 45,000 tonnes of Se are released into the environment by anthropogenic activities. Today, bioremediation is emerging as a promising strategy for the immobilisation of this element in the environment. In the case of Se, the enzymatic reduction of Se(IV) and Se(VI) to Se(0) could be one of the most effective, green and circular economy strategy for biorremediation. The strain Stenotrophomonas bentonitica BII-R7 has been extensively characterised for its ability to reduce Se(VI) and Se(IV) to Se(0), with subsequent structural transformation of the oxidation products (SeNPs). Proteomics and transcriptomics studies identified different proteins involved in the Se reduction. The present study describes the cellular mechanisms used by this bacterium to mitigate Se toxicity using a multidisciplinary approach combining flow cytometry, microscopy and spectroscopy.

Our results showed that whilst cell viability decreased with increasing Se(IV) concentrations, membrane potential and intracellular ROS generations increased linearly to metal concentration, reaching a maximum between 48-72h at 2 mM. However, Se(VI) toxicity showed its maximum effect at 68h on the cell viability and membrane potential at a concentration of 200 mM, while at 50 mM no toxic effect was observed. STEM analysis showed time dependent SeNPs cellular localization. Intracellular amorphous SeNPs were observed after 24h of incubation at 2 mM Se(IV) concentration. Later, extracellular crystalline nanostructures were appreciated between 72-144h incubating at the same Se(IV) concentration. In the case of Se(VI), no reduction products were found at 50 mM, while at 200 mM, mostly intracellular nanotubes with a crystalline structure were identified at 24-48h.

The results obtained indicate that S. bentonitica BII-R7 has a great potential for bioremediation and nanotechnology, given its ability to reduce the oxidised forms of Se to Se(0) with subsequent transformation of the amorphous nanoparticles to crystalline nanostructures.