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Enzyme Involvement in the Biosynthesis of Selenium Nanoparticles by Geobacillus wiegelii Strain GWE1 Isolated from a Drying Oven

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Abstract : The biosynthesis of nanoparticles by microorganisms, on the contrary to chemical synthesis, is an environmentally-friendly process which has low energy requirements. In this investigation, we used the microorganism Geobacillus wiegelii, strain GWE1, an aerobic thermophile belonging to genus Geobacillus, isolated from a drying oven. This microorganism has the ability to reduce selenite evidenced by the change of color from colorless to red in the culture. Elemental analysis and composition of the particles were verified using transmission electron microscopy and energy-dispersive X-ray analysis. The nanoparticles have a defined spherical shape and a selenium elemental state. Previous experiments showed that the presence of the whole microorganism for the reduction of selenite was not necessary. The results strongly suggested that an intracellular NADPH/NADH-dependent reductase mediates selenium nanoparticles synthesis under aerobic conditions. The enzyme was purified and identified by mass spectroscopy MALDI-TOF TOF technique. The enzyme is a 1-pyrroline-5-carboxylate dehydrogenase. Histograms of nanoparticles sizes were obtained. Size distribution ranged from 40-160 nm, where 70% of nanoparticles have less than 100 nm in size. Spectroscopic analysis showed that the nanoparticles are composed of elemental selenium. To analyse the effect of pH in size and morphology of nanoparticles, the synthesis of them was carried out at different pHs (4.0, 5.0, 6.0, 7.0, 8.0). For thermostability studies samples were incubated at different temperatures (60, 80 and 100 ºC) for 1 h and 3 h. The size of all nanoparticles was less than 100 nm at pH 4.0; over 50% of nanoparticles have less than 100 nm at pH 5.0; at pH 6.0 and 8.0 over 90% of nanoparticles have less than 100 nm in size. At neutral pH (7.0) nanoparticles reach a size around 120 nm and only 20% of them were less than 100 nm. When looking at temperature effect, nanoparticles did not show a significant difference in size when they were incubated between 0 and 3 h at 60 ºC. Meanwhile at 80 °C the nanoparticles suspension lost its homogeneity. A change in size was observed from 0 h of incubation at 80ºC, observing a size range between 40-160 nm, with 20% of them over 100 nm. Meanwhile after 3 h of incubation at size range changed to 60-180 nm with 50% of them over 100 nm. At 100 °C the nanoparticles aggregate forming nanorod structures. In conclusion, these results indicate that is possible to modulate size and shape of biologically synthesized nanoparticles by modulating pH and temperature.

Keywords : genus Geobacillus, NADPH/NADH-dependent reductase, selenium nanoparticles, biosynthesis
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