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## ACCEPTED MANUSCRIPT

Effect of selenite and selenium nanoparticles on lactic bacteria. A multi-analytical study.

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#### Abstract

The effect of selenite and chitosan-modified SeNPs (CS-SeNPs) on Lactobacillus bulgaricus was evaluated through a multianalytical approach based on flow cytometry and transmission electron microscopy (TEM), and high performance liquid chromatography (HPLC) on line coupled to inductively coupled plasma mass spectrometry (ICP-MS) to investigate both lactic bacteria viability in presence of selenium compounds and transformation of selenium compounds once accumulated by the bacteria. For this purpose, L. bulgaricus were grown at 37 °C for 24, 48 and 72 h in presence of 1 and 10 µg Se·mL<sup>-1</sup> of selenium as CS-SeNPs and Na<sub>2</sub>SeO<sub>3</sub> . No significant differences in bacteria cell viability between selenium-enriched and control bacteria were observed when adding 1 µg Se·mL<sup>-1</sup> either as Se(IV) or CS-SeNPs. In contrast, bacteria viability decreases when increasing selenium concentration up to 10 ug Se·mL<sup>-1</sup> being this effect more accused when selenium was supplemented as selenite. Under these conditions SeNPs killed approximately 20% of Lactobacillus after 24 hours of exposure while the percentage increase up to 60% of bacteria when 10 µg Se·mL<sup>-1</sup> as selenite was applied. Transmission electron microscopy (TEM) images show that CS-SeNPs readily enter in the bacteria cells preserving membrane integrity and therefore causing no cellular damage. Results from HPLC-ICPMS indicate that most of the CS-SeNPs in the culture media were transformed by the bacteria to organo

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