Ultrastructural characterization of Membrane Vesicles (MVs) produced by *Lactobacillus reuteri*.

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Nanotechnology represents a great promise for drug delivery opening new therapeutic opportunities for agents that cannot be effectively used for therapeutic use. In fact, nanomedicine has recently received increasing attention for its ability to improve the efficacy of cancer therapeutics, imaging tools, antibacterial agents and gene delivery vesicles (1, 2, 3).
As a natural phenomenon, bacteria release Membrane Vesicles (MVs) at a various stages of growth, under environmental conditions, or in response to chemical signals. MVs are a demonstrated form of communication used by bacteria.
Since the probiotic Gram positive *Lactobacillus reuteri* DSM 17938 develops biofilm *in vitro* producing factors which give health benefit to the host, the aim of this study was the ultrastuctural characterization of MVs produced from *L. reuteri* planktonic (pMVs) and biofilm (bMVs) phenotypes.
The microorganism is capable of generating MVs in both planktonic and biofilm phenotypes. *L. reuteri* DSM 17938 biofilm formation was evaluated by syto 9 staining and Confocal Laser Scanning Microscopy (CLSM) analysis. The data obtained in this study demonstrated that *L .reuteri* developed a well-structured biofilm after 24 h of incubation. Moreover, MVs production, in the two phenotypes, was confirmed by Scanning Electron Microscopy (SEM) observations. In order to evaluate MVs biological composition (as lipids and proteins), pMVs and bMVs were isolated by filtration and ultracentrifugation and subsequently submitted at an enzymatic digestion with DNase I, Proteinase K and Phospholipase C and finally analyzed by Transmission Electron Microscopy (TEM) and SEM.
The structure and composition of MVs may provide a relevant information about the use of such structures in the development of vesicles-based therapeutic systems. The analysis performed by TEM and SEM demonstrated that there are interesting differences both in the ultrastructural organization and in dimensions between pMVs and bMVs .

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