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## OMVs from Novosphingobium sp. PP1Y: isolation, purification and partial characterization of a potential biotechnological tool for biocatalysis and drug delivery

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Outer membrane vesicles (OMVs) are nanostructures of 20-200 nm diameter deriving from the surface of several Gram-negative bacteria as part of their natural growth cycle, which are involved in cellular communication, biofilm formation and nutrient acquisition. OMVs have attracted the attention of biotechnological industries for their potential use as immobilization tools and drug delivery systems. The biotechnological use of OMVs is currently limited, among others, by the presence of the immunogenic LPS typically present in the outer membrane (OM) of Gram-negative bacteria. Novosphingobium sp. PP1Y is a marine microorganism belonging to the order Sphingomonadales, which lack LPS on their OM, which was isolated by our research group in the harbor of Pozzuoli and microbiologically characterized; its genome has been sequenced and completely annotated. We have successfully isolated OMVs from Novosphingobium sp. PP1Y grown in minimal medium. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) were used to confirm OMVs production, which resulted to occur only when PP1Y was grown in minimal medium supplemented with 0.4% glutamic acid as sole carbon and energy source. AFM, DLS and nanosize analysis of purified vesicles showed these OMVs to have a circular morphology and a diameter of  $\approx$ 200 nm. A finely controlled fatty acids and proteins distribution in these extracellular nanostructures was found. Interestingly, the presence of an active protease IV activity was verified in purified OMVs from strain PP1Y, thus prompting a future use of these vesicles as biocatalysts and/or as drug delivery systems in biomedical applications.

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