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Isolation and Characterization of Outer Membrane Vesicles from *Novosphingobium* sp. PP1Y: a novel biotechnological tool for biocatalysis and drug delivery?

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Outer membrane vesicles (OMVs) are nanoscale proteoliposomes of 20-200 nm diameter, derived from the surface of many gram-negative and gram-positive bacteria as part of their natural growth cycle. Natural OMVs have several important roles; for example, they can contribute to bacterial survival by reducing levels of toxic compounds, removing stress products from the cell such as misfolded periplasmic proteins, or are involved in biofilm formation. Interest in these bioparticles has grown over the years, leading to important highlights concerning their composition, production, and their role in cellular communication and environmental adaptation. In this context, an appealing source of OMVs can be retrieved in *Novosphingobium* sp. PP1Y, a marine microorganism isolated in a polluted area of the harbor of Pozzuoli (Naples, Italy) and microbiologically characterized by our research group. This bacterium is a non-pathogenic microorganism belonging to the order Sphingomonadales, which is endowed with the ability to grow on a wide array of mono- and polycyclic aromatic compounds (PAHs) used as sole carbon and energy source. *N. sp.* PP1Y genome has been sequenced and completely annotated, showing the presence of a complex metabolic pattern, responsible for its growth in harsh environmental conditions. The growth of strain PP1Y shows the presence of an equilibrium between a sessile and a planktonic form. We have successfully isolated OMVs from *N. sp.* PP1Y grown in minimal medium supplemented with 0.4% glutamate as sole carbon and energy source (Fig.1). AFM, DLS and Nanosize analysis on purified vesicles showed that *N. sp.* PP1Y OMVs have a circular morphology with a diameter of ≈ 200 nm and are uniform in size and shape, in accordance with other OMVs described in literature. Proteomic and fatty acid analysis suggested a specific protein and fatty acid fingerprint of these extracellular nanostructures; in particular, among all proteins identified in OMVs, Protease IV resulted to be the more abundant. Preliminary analysis showed that Protease IV is present in OMVs from strain PP1Y in its active form, thus paving the way to the use of these structures as vehicles of specific enzymatic activities of biotechnological importance.

Recent Publications:

1. De Lise F, Mensitieri F, Tarallo V, Ventimiglia N, Vinciguerra R, Tramice A, Marchetti R, Pizzo E, Notomista E, Cafaro V, Molinaro A, Birolo L, Di Donato A, Izzo V (2016) RHA-P: isolation, expression and characterization of a bacterial α -L-rhamnosidase from *Novosphingobium* sp. PP1Y. J. Mol. Catal. B: Enzym 134, 136-147.
2. Donadio G, Sarcinelli C, Pizzo E, Notomista E, Pezzella A, Di Cristo C, De Lise F, Di Donato A, Izzo V (2015) The Toluene o-Xylene Monooxygenase Enzymatic Activity for the Biosynthesis of Aromatic Antioxidants. PLoS One 10 (4), e0124427. doi: 10.1371/journal.pone.0124427.
3. D'Argenio V, Notomista E, Petrillo M, Cantiello P, Cafaro V, Izzo V, Naso B, Cozzuto L, Durante L, Troncone L, Paoletta G, Salvatore F, Di Donato A (2014) Complete sequencing of *Novosphingobium* sp. PP1Y reveals a biotechnologically meaningful metabolic pattern. BMC Genomics 15:384.
4. Izzo V, Tedesco P, Notomista E, Pagnotta E, Di Donato A, Trincone A, Tramice A (2014) α -Rhamnosidase activity in the marine isolate *Novosphingobium* sp. PP1Y and its use in the bioconversion of flavonoids. J. Mol. Catal. B: Enzym. 105, 95-103.

Biography

Dr. Viviana Izzo has completed her PhD in Biochemistry and Molecular Biology at the age of 26 years from the University Federico II of Naples. From 2004 through 2006, Dr. Izzo has been postdoc Associate at the Department of Chemistry at the Massachusetts Institute of Technology (MIT, Cambridge, MA, USA). She is currently Assistant Professor of Biochemistry at the University of Salerno, and is responsible for the Unit of Pharmacokinetics at the University Hospital "San Giovanni di Dio e Ruggi d'Aragona" of Salerno (Italy). Dr. Izzo benefits of several national and international collaborations and is referee for several peer-reviewed scientific journals of Biochemistry, Biotechnology and Applied Microbiology. Dr. Izzo bears an expertise in a range of biochemical, molecular biology and microbiological subjects and techniques gained through both academic study and recent work experience.

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