Membrane Vesicles Play a Role in Metal-Microbe Interactions

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Membrane vesicles (MV) are extracellular formations derived from the outer-membrane of Gram-negative bacteria. Their biological roles include transport of virulence factors, protein and DNA exchange, cell-cell communication and biofilm formation. Although it is known that metals interact with the bacterial membrane, how MVs modulate this interaction is unknown. The Gram-negative bacterium *Burkholderia vietnamiensis* $PR1_{301}$ (PR1) has been used as a model microorganism to study metal-microbe interactions and is 20-fold more resistant to Zn at pH 5 versus pH 7. The mechanism of pH-dependent metal resistance in PR1 has not been identified. Recently, we have found that MVs may be playing a role in metal-microbe interactions. When PR1 was grown in the presence of 250 mg L^{-1} Zn at pH 6, Zn was found localized to MVs but not cells. For this reason, we are investigating the possible involvement of MVs in pH-dependent metal resistance in PR1. To accomplish this, first we developed a method using filtration and differential centrifugation to isolate and quantify MV production in PR1. At pH 6, it was found that a maximum production of MVs (3 μg protein mL^{-1} or 2 % of total culture protein) occurred at early stationary phase. This sample point will be used to sample for MV production at pH 5 and 7 (with and without sub-lethal Zn concentrations) to evaluate differential MV production and characterize their protein content and fatty acid composition. Additionally, field-flow fractionation (FFF) coupled to static light scattering was used to fractionate MVs and determine their geometric radius and absolute number. This was conducted at pH 6 with and without 75 mg L^{-1} Zn. Results demonstrate that relative to cells unamended with Zn, MVs produced when PR1 was exposed to Zn are 10 ± 2.8 % larger and 45 ± 21 % less abundant during mid-exponential phase growth, though at late stationary phase they are not statistically different. Further analysis of collected MV fractions will be done using inductively coupled plasma mass spectrometry (ICP-MS) to quantify changes in Zn content. The results of these studies will demonstrate whether MVs containing Zn are a novel mechanism of metal resistance or whether they harm the microorganism by allowing Zn to be more bioavailable to the microorganism. This will highlight the significance of a key overlooked aspect of metal-microbe interactions.

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